Patent Office
Canberra

I, KIM MARSHALL, MANAGER EXAMINATION SUPPORT AND SALES, hereby certify that the annexed is a true copy of the Provisional specification in connection with Application No. PO 9108 for a patent by COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION and THE AUSTRALIAN NATIONAL UNIVERSITY filed on 12 September 1997.

I further certify that the annexed specification is not, as yet, open to public inspection.

PRIORITY DOCUMENT

WITNESS my hand this Seventeenth
day of September 1998

KIM MARSHALL
MANAGER EXAMINATION SUPPORT AND SALES

AUSTRALIA

Patents Act 1990

PROVISIONAL SPECIFICATION

Applicant(s): COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION
and
THE AUSTRALIAN NATIONAL UNIVERSITY

Invention Title: REGULATION OF GENE EXPRESSION IN PLANTS

The invention is described in the following statement:

REGULATION OF GENE EXPRESSION IN PLANTS

This invention relates to methods of modulating the expression of desired genes in plants, and to DNA sequences and genetic constructs for use in these methods. In one preferred embodiment, the invention relates to methods and constructs for targeting of expression specifically to the endosperm of the seeds of cereal plants such as wheat, and for modulating the time of expression in the target tissue. This is achieved by the use of promoter sequences from enzymes of the starch biosynthetic pathway. In preferred embodiments of the invention, the sequences and/or promoters are those of starch branching enzyme I, starch branching enzyme II, soluble starch synthase, and starch debranching enzyme, all derived from *Triticum tauschii*, the D genome donor of hexaploid bread wheat.

BACKGROUND OF THE INVENTION

Starch is an important constituent of the cereal grains and of flours, accounting for about 65-67% of the weight of the grain at maturity . It is produced in the amyloplast of the grain endosperm by the concerted action of a number of enzymes, including ADP-Glucose pyrophosphorylase (EC 2.7.7.27), starch synthases (EC 2.4.1.21), branching enzymes (EC 2.4.1.18) and debranching enzymes (EC 3.2.1.41 and EC 3.2.1.68) (Ball *et al*, 1996; Martin and Smith, 1995; Morell *et al*, 1995). Some of the proteins involved in the synthesis of starch can be recovered from the starch granule (Denyer *et al*, 1995; Rahman *et al*, 1995).

Most wheat cultivars normally produce starch containing 25% amylose and 75% amylopectin. Amylose is composed of large linear chains of α (1-4) linked α-D-glucopyranosyl residues, whereas amylopectin is a branching form of α-glycan linked by α (1-6) linkages. The ratio of amylose and amylopectin, the branch chain length and the

number of branch chains of amylopectin are the major factors which determine the properties of wheat starch.

Wheat contains three sets of chromosomes (A, B and D) in its very large genome of about 10^{10} bp. The donor of the D genome to wheat is *Triticum tauschii*, and by using a suitable accession of this species the genes from the D genome can be studied separately (Lagudah *et al*, 1991).

Starch with various properties has been widely used in industry, food science and medical science. High amylose wheat can be used for the plastic substitutes and in paper manufacture to protect the environment; in health foods to reduce bowel cancer and heart disease; and in sport food to improve the athletes' performance. High amylopectin wheat may be suitable for Japanese noodles and is used as a thickener in the food industry.

There is comparatively little variation in starch structure found in wheat varieties, because the hexaploid nature of wheat prevents mutations from being readily identified. Dramatic alterations in starch structure are expected to require the combination of homozygous recessive alleles from each of the 3 wheat genomes, A, B and D. This requirement renders the probability of finding such mutants in natural or mutagenised populations very low. Variation in wheat starch is desirable in order to enable better tailoring of wheat starches for processing and end-user requirements.

Key commercial targets for the manipulation of starch biosynthesis are:

1. "Waxy" wheats in which amylose content is decreased to insignificant levels. This outcome is expected to be obtained by eliminating granule-bound starch synthase activity.

2. High amylose wheats, expected to be obtained by suppressing starch branching enzyme-II activity.

3. Wheats which continue to synthesise starch at elevated temperatures, expected to be obtained by identifying or introducing a gene encoding a heat stable soluble starch synthase.

4. "Sugary types" of wheat which contain increased amylose content and free sugars, expected to be obtained by manipulating an isoamylase-type debranching enzyme.

There are two general strategies for obtaining wheats with altered starch structure:

(a) using genetic engineering strategies to suppress the activity of a specific gene, or to introduce a novel gene into a wheat line; and

(b) selecting among existing variation in wheat for missing (or "null") or altered alleles of a gene in each of the genomes of wheat, and combining these by plant breeding.

Branching enzymes are involved in the production of glucose α (1,6) branches. Of the two main constituents of starch, amylose is essentially linear, but amylopectin is highly branched; thus branching enzymes are thought to be directly involved in the synthesis of amylopectin but not amylose. There are two types of branching enzymes in plants ,starch branching enzyme I (SBE I) and starch branching enzyme II (SBE II), and both are about 85 kDa in size. At the nucleic acid level there is about 65% sequence identity between types I and II in the central portion of the molecules; the sequence identity between SBE I from different cereals is about 85% overall (Burton *et al*, 1995; Morell *et al*, 1995).

In cereals, SBE I genes have so far been reported only for rice (Kawasaki *et al*, 1991) and wheat (Rahman *et al*, 1997). A cDNA sequence for wheat SBE I is available on the Genbank database. As far as we are aware, no promoter sequence for wheat SBE I has been reported. We have characterised an SBE I gene (called *wSBE I-D2*) from *Triticum tauschii* (the donor of the D genome to wheat),

that encoded a novel protein sequence in that it was missing approximately 65 amino acids at the C-terminal end, and appeared not to contain some of the conserved amino acid motifs characteristic of this class of enzyme (Svensson, 1994). Although *wSBE I-D2* was expressed as mRNA, no corresponding protein has yet been found in our analysis of SBE I isoforms from the endosperm, and thus it is possible that this gene is a transcribed pseudogene.

Genes for SBE II are less well characterised; the only one for cereals which has been fully characterised is that of rice (Kawasaki *et al*, 1993). A cDNA sequence for SBE II from wheat is available on the Genbank database; although the sequences are very similar to those reported herein there are differences near the N-terminal of the protein, which specifies its intracellular location. No promoter sequences have been reported, as far as we are aware.

Wheat granule-bound starch synthase (GBSS) is responsible for amylose synthesis, while wheat branching enzymes together with soluble starch synthases are considered to be directly involved in amylopectin biosynthesis. A number of isoforms of soluble and granule-bound starch synthases have been identified in developing wheat endosperm (Denyer *et al*, 1995). There are three distinct isoforms of starch synthases, 60 kDa, 75 or 77 kDa and 100-105 kDa, which exist in the starch granules (Denyer *et al*, 1995; Rahman *et al*, 1995). The 60 kDa GBSS is the product of the wx gene. The 75 or 77 kDa protein is a wheat soluble starch synthase (SSS) which is present in both the soluble fraction and the starch granule-bound fraction of the endosperm. However, the 100-105 kDa proteins, which are another type of soluble starch synthase, are located only in starch granules (Denyer *et al*, 1995; Rahman *et al*, 1995). To our knowledge there has been no report of any complete wheat SSS sequence, either at the protein or the nucleotide level.

Both cDNA and genomic DNA encoding a soluble starch synthase I of rice have been cloned and analysed (Baba *et al*, 1993; Tanaka *et al*, 1995). The cDNAs encoding potato soluble starch synthase SSII and SSIII and pea soluble starch synthase SSII have also been reported (Edwards *et al*, 1995; Marshall *et al*, 1996; Gernot *et al*, 1996; Dry *et al*, 1992). However, corresponding cDNA sequences for wheat have hitherto not been available, and the genes for other enzymes involved in starch biosynthesis have not previously been isolated.

Approach (b) referred to above has been demonstrated for the gene granule bound starch synthase. Null alleles on chromosomes 7A, 7D and 4A were identified by the analysis of GBSS protein bands by electrophoresis, and combined by plant breeding to produce a wheat line containing no GBSS, and no amylose. Subsequently, PCR-based DNA markers have been identified, which also identify null alleles for the GBSS loci on each of the three wheat genomes.

SUMMARY OF THE INVENTION

In this application we report the isolation and identification of novel genes from *T. tauschii*, the D-genome donor of wheat, that encode SBE I, SBE II, a 75 kDa SSS, and an isoamylase-type debranching enzyme (DBE). Because of the very close relationship between *T. tauschii* and wheat, as discussed above, results obtained with *T. tauschii* can be directly applied to wheat with little if any modification. Such modification as may be required represents routine trial and error experimentation. Sequences from these genes can be used as probes to identify null or altered alleles in wheat, which can then be used in plant breeding programes to provide modifications of starch characteristics. The novel sequences of the invention can be used in genetic engineering strategies or to introduce a desired gene into a host plant, to provide antisense sequences for

suppression of one or more specific genes in a host plant, in order to modify the characteristics of starch produced by the plant.

In its most general aspect, the invention provides a nucleic acid sequence encoding an enzyme of the starch biosynthetic pathway in a cereal plant, said enzyme being selected from the group consisting of starch branching enzyme I, starch branching enzyme II, starch soluble synthase, and debranching enzyme, with the proviso that the enzyme is not soluble starch synthase I of rice, or starch branching enzyme I of rice or maize.

Preferably the nucleic acid sequence is a DNA sequence, and may be genomic DNA or cDNA. Preferably the sequence is one which is functional in wheat. More preferably the sequence is derived from *Triticum* species, most preferably *Triticum tauschii*.

Where the sequence encodes soluble starch synthase, preferably the sequence encodes the 75 kD soluble starch synthase of wheat.

Biologically-active untranslated control sequences of genomic DNA are also within the scope of the invention. Thus the invention also provides the promoter of an enzyme as defined above.

In a preferred embodiment of this aspect of the invention, there is provided a genetic construct comprising a nucleic acid sequence of the invention, a biologically-active fragment thereof, or a fragment thereof encoding a biologically-active fragment of an enzyme as defined above, operably linked to one or more nucleic acid sequences facilitating expression of said enzyme in a plant, preferably a cereal plant. The construct may be a plasmid or a vector, preferably one suitable for use in transformation of plant. Such a suitable vector is a bacterium of the genus *Agrobacterium*, preferably *Agrobacterium tumefacens*. Methods of transforming cereal plants using *Agrobacterium tumefaciens* are known; see for

example Australian Patent No. 667939 by Japan Tobacco Inc., and Tingay *et al* (1997).

In a second aspect, the invention provides a genetic construct for targeting of a desired gene to endosperm of a cereal plant, and/or for modulating the time of expression of a desired gene in endosperm of a cereal plant, comprising one or more promoter sequences selected from SBE I promoter, SBE II promoter, SSS promoter, and DBE promoter, operatively linked to a nucleic acid sequence encoding a desired protein, and optionally also operatively linked to one or more additional targeting sequences and/or one or more 3' untranslated sequences.

The nucleic acid encoding the desired protein may be in either the sense orientation or in the antisense orientation. Preferably the desired protein is an enzyme of the starch biosynthetic pathway. For example, the antisense sequences of GBSS, starch debranching enzyme, SBE II, low molecular weight glutenin, or grain softness protein I, may be used. Preferred sequences for use in sense orientation include those of bacterial isoamylase, bacterial glycogen synthase, or wheat high molecular weight glutenin Bx17. It is contemplated that any desired protein which is encoded by a gene which is capable of being expressed in the endosperm of a cereal plant is suitable for use in the invention.

In a third aspect, the invention provides a method of modifying the characteristics of starch produced by a plant, comprising the step of:

(a) introducing a gene encoding a desired enzyme of the starch biosynthetic pathway into a host plant, and/or

(b) introducing an anti-sense nucleic acid sequence directed to a gene encoding an enzyme of the starch biosynthetic pathway into a host plant,

wherein said enzymes are as defined above.

Where both steps (a) and (b) are used, the enzymes in the two steps are different.

Preferably the plant is a cereal plant, more preferably wheat or barley.

As is well known in the art, anti-sense sequences can be used to suppress expression of the protein to which the anti-sense sequence is complementary. It would be evident to the person skilled in the art that different combinations of sense and anti-sense sequences may be chosen so as to effect a variety of different modifications of the characteristics of the starch produced by the plant.

In a fourth aspect, the invention provides a method of targeting expression of a desired gene to the endosperm of a cereal plant, comprising the step of transforming the plant with a construct according to the invention.

According to a fifth aspect, the invention provides a method of modulating the time of expression of a desired gene in endosperm of a cereal plant, comprising the step of transforming the plant with a construct according to the second aspect of the invention.

Where expression at an early stage following anthesis is desired, the construct preferably comprises the SBE II promoter. Where expression at a later stage following anthesis is desired, the construct preferably comprises the SBE I promoter.

In a sixth aspect, the invention provides a method of identifying a null or altered allele encoding an enzyme of the starch biosynthetic pathway, comprising the steps of subjecting DNA from a plant suspected to possess such an allele to a DNA fingerprinting assay, wherein DNA probes used in the assay comprise one or more of the nucleic acid sequences of the invention. The nucleic acid sequence may be a genomic DNA or a cDNA, and may comprise the full-length coding sequence or a fragment thereof.

DNA fingerprinting methods are well known in the art, and any suitable technique may be used.

While the invention is described in detail in relation to wheat, it will be clearly understood that it is

also applicable to other cereal plants of the family Gramineae, such as maize, barley and rice.

Methods for transformation of monocotyledonous plants such as wheat, maize, barley and rice and for regeneration of plants from protoplasts or immature plant embryos are well known in the art. See for example Lazzeri *et al*, 1991; Jahne *et al*, 1991 and Wan and Lenaux, 1994 for barley; Wirtzens *et al*, 1997; Tingay *et al*, 1997; Canadian Patent Application No. 2092588 by Nehra; Australian Patent Application No. 61781/94 by National Research Council of Canada, and Australian Patent No. 667939 by Japan Tobacco Co.

The sequences of ADP glucose pyrophosphorylase from barley (Australian Patent Application No. 65392/94), starch debranching enzyme and its promoter from rice (Japanese Patent Publication No. Kokai 6261787 and Japanese Patent Publication No. Kokai 5317057), and starch debranching enzyme from spinach and potato (Australian Patent Application No. 44333/96) are all known.

Detailed Description of the Drawings

The invention will be described in detail by reference only to the following non-limiting examples and to the figures.

Figure 1 shows the hybridisation of genomic clones isolated from *T. tauschii*.

DNA was extracted from the different clones, digested with *BamHI* and hybridised with the 5' end of the maize SBE I cDNA. Lanes 1, 2, 3 and 4 correspond to DNA from clones λE1, λE2, λE6 and λE7 respectively. Note that clones λE1 and λE2 give identical patterns, the SBE I gene in λE6 is a truncated form of that in λE1, and λE7 gives a clearly different pattern.

Figure 2 shows the hybridisation of DNA from *T. tauschii*.

DNA from *T. tauschii* was digested with *BamHI* and the hybridisation pattern compared with DNA from λE1 and

λE7 digested with the same enzyme. Fragment E1.1 (see Figure 3) from λE1 was used as the probe; it contains some sequences that are over 80% identical to sequences in E7.8. Approximately 25 µg of *T. tauschii* DNA was electrophoresed in lane 1, and 200 pg each of λE1 and λE7 in lanes 2 and 3, respectively.

Figure 3 shows the restriction maps of clone λE1 and λE7. The fragments obtained with EcoRI and BamHI are indicated. The fragments sequenced from λE1 are E1.1, E1.2, a part of E1.7 and a part of E1.5.

Figure 4 shows the nucleotide sequence of part of wSBE I-D4, and the deduced amino acid sequence and N-terminal sequence of SBE I (Morell *et al*, 1997).

Figure 5 shows the hybridisation of SBE I genomic clones with the following probes,

A. wSBE I-D45 (derived from the 5' end of the gene and including sequence from fragments E1.1 and E1.7), and

B. wSBE I-D43 (derived from the 3' end of the gene and containing sequences from fragment E1.5).

Clones λE7 and λE22 do not hybridise to either of the probes, but do hybridise with a probe from E1.1. Clone λE30 contains a sequence unrelated to SBE I.

Figure 6 shows the intron-exon structure of wSBE I-D4 compared to the corresponding structures of rice SBE I (Kawasaki *et al*, 1993) and wSBE I-D2 (Rahman *et al*, 1997). The intron-exon structure of wSBE I-D4 is deduced by comparison with the major species of wSBE I-D4 cDNA present in the endosperm.

The dark rectangles correspond to exons and the light rectangles correspond to introns. The bars above the structures indicate the percentage identity in sequence between the indicated exons and introns of the relevant genes. Note that intron 2 shares no signifcant sequence identity and is not indicated.

Figure 7 shows the alignment of cDNA clones to obtain the sequence represented by wSBE I-D4 cDNA. BED4

and BED5 were obtained from screening the cDNA library with maize BEI (Baba *et al*, 1991). BED1, 2 and 3 were obtained by RT-PCR using defined primers.

Figure 8 shows the amino acid sequence of SBE I as deduced from the sequence of wSBE I-D4 cDNA. The N-terminal sequence of SBE I (Morell *et al*, 1997) is in bold, and residues considered by Svensson (1994) to be invariant in the α-amylase family are underlined.

Figure 9 shows the comparison of deduced amino acid sequence of wSBE I-D4 cDNA with the deduced amino acid sequence of rice SBE I (Nakamura *et al*, 1992), maize SBE I (Baba *et al*, 1991), wSBE I-D2 type cDNA (Rahman *et al*, 1997), pea SBE II (equivalent to maize SBE I, Burton *et al*, 1995), and potato SBE I (Cangiano *et al*, 1993). Residues present in at least three of the sequences are identified in the consensus sequence in capitals.

Figure 10 shows the expression of SBE I type sequences during endosperm development. The probe used was wSBE I-D43C, corresponding to the untranslated 3' end of wSBE I-D4 cDNA. There is no hybridisation to RNA extracted from leaves or florets prior to anthesis.

Figure 11 shows the comparison of wSBE I-D4 and rice SBE I genomic sequence (Kawasaki *et al*, 1993; Accession Number D10838) using the programs Compares and DotPlot (Devereaux *et al*, 1984). The programs used a window of 21 bases with a stringency of 14 to register a dot.

Figure 12 shows the hybridisation of wheat DNA from chromosome-engineered lines using the following probes:

A. wSBE I-D45 (from the 5' end of the gene),

B. wSBE I-D43 (from the 3' end of the gene), and

C. wSBE I-D4R (repetitive sequence approximately 600 bp 3' to the end of wSBE I-D4 sequence.

N7AT7B, no 7A chromosome, four copies of 7B chromosome; N7BT7D, no 7B chromosome, four copies of 7D

chromosome; NTDT7A, no 7D chromosome, four copies of 7A chromosome. The chromosomal origin of hybridising bands is indicated.

Figure 13 shows the entire sequence of the wSBE I-D4 gene. The promoter sequences is given in (a) up to the first translated amino acid. The coding sequence of the gene is given in (b), with about 50 bases of the promoter sequence.

Figure 14 shows the sequence of wSBE I-D43C, representing the 3' untranslated region of wSBE I-D4cDNA.

Figure 15 shows the hybridisation of genomic clones F1, F2, F5 and F6 with the entire SBE-9 sequence. The DNA from the clones was purified and digested with either BamHI or EcoRI, separated on agarose, blotted onto nitrocellulose and hybridised with labelled SBE-9 (a SBE II type cDNA). The pattern of hybridising bands is different in the four isolates.

Figure 16a shows the N-terminal sequence of SBE II from wheat as in Morell *et al*, (1997).

Figure 16b shows the deduced amino acid sequence from part of wSBE II-D1 that encodes the N-terminal sequence as described in Morell *et al*, (1997).

Figure 16c shows the deduced amino acid sequence from SBE-9 (a SBE II type cDNA).

Figure 17 shows the deduced exon-intron structure for a part of wSBE II-D1. The scale is marked in bases. The dark rectangles are exons.

Figure 18 shows the hybridisation of DNA from chromosome engineered lines of wheat (cultivar Chinese Spring) with a probe from nucleotides 550-850 from SBE-9. The band of approximately 2.2 kb are missing in the line in which missing chromosome 2D is absent.

T2BN2A: four copies of chromosome 2B, no copies of chromosome 2A;

T2AN2B: four copies of chromosome 2A, no copies of chromosome 2B;

T2AN2D: four copies of chromosome 2A, no copies of chromosome 2D.

Figure 19 shows the entire sequence of the wSBE II-D1 gene. The promoter sequence is given in (a) up to the first translated amino acid, and the coding sequence of the gene is given in (b).

Figure 20a shows the N-terminal sequence of SSS protein isolated from starch granules (Rahman *et al*, 1995) and deduced amino acid sequence of part of Sm2.

Figure 20b shows the nucleotide sequence of cDNA clone (sm2) for wheat soluble starch synthase.

Figure 20c shows the nucleotide sequence of genomic clone for SSS.

Figure 21 shows the deduced amino acid sequence of cDNA clone (sm2) for SSS.

Figure 22 shows the hybridisation of genomic clones sg1, 3, 4, 6 and 11 with the cDNA clone (sm2) for SSS. DNA was purified from indicated genomic clones, digested with BamHI or SacI and hybridised to sm2. Note that the hybridisation patterns for sg1, 3 and 4 are clearly different from each other.

Figure 23 shows the hybridisation of RNA from wheat tissues with the cDNA clone for SSS (sm2) as well as indicated regions of SBE II and SBE I. The tissues indicated are leaves, florets and endosperm 5-8, 10-15 and 18-22 days after anthesis (glasshouse grown material).

Figure 24 shows the hybridisation of DNA from chromosome engineered lines of wheat (cultivar Chinese Spring) digested with PvuII, with the sm2 probe.

N7AT7B: no 7A chromosome, four copies of 7B chromosome;

N7BT7D: no 7B chromosome, four copies of 7D chromosome;

N7DT7A: no 7D chromosome, four copies of 7A chromosome.

A band is missing in the N7BT7A line.

Figure 25 shows the promoter sequence of soluble

starch synthase from wheat endosperm. The sequence up to the first encoded methionine (codon ATG) is included.

Figure 26 shows the comparison of wheat and rice soluble starch synthase genomic sequences. The dark rectangles indicate exons and the light rectangles represent introns. The break indicates the area where sequencing needs to be completed.

Figure 27a shows the DNA sequence of a portion of the wheat debranching enzyme (WDBE-1) PCR product. The PCR product was generated from wheat genomic DNA (cultivar Rosella) using primers based on sequences conserved in debranching enzymes from maize and rice.

Figure 27b shows a comparison of the nucleotide sequence of wheat debranching enzyme I (WDBE-I) PCR fragment with the maize *Sugary-1* sequence.

Figure 28 shows the Southern blotting of *T. tauschii* DNA with DBE PCR product. DNA from *T. tauschii* was digested with BamHI , electrophoresed, blotted and hybridised to DBE PCR product. A band of approximately 2 kb hybridised.

Figure 29 illustrates the design of 9 intron spanning BE II primer sets. Primers were based on wSBE II-D1 sequence (Figure 19) and were designed such that intron sequences in the wSBE II-D1 sequence (deduced from Figure 17) were amplified by PCR.

Figure 30 shows the results of SBE II-Intron 6 primer set on chromosome 2 nullisomic :tetrasomic lines of the wheat cultivar Chinese Spring.

BBD: tetra 2B nulli 2A;

AAD: tetra 2A nulli 2B;

AAB: tetra 2A nulli 2D;

CS Chinese Spring normal;

ADD: tetra 2D nulli 2B;

ABB: tetra 2B nulli 2D;

AABB: tetraploid wheat having only the A and B genomes.

The horizontal axis indicates the size of the product.

Figure 31 shows the results obtained using the SBE II-Intron 6 primer set on the wheat varieties (a) Chinese Spring and (b) Rosella.

Example 1 Identification of Gene Encoding SBE I

Construction of Genomic Library and Isolation of Clones

The genomic library used in this study was constructed from *Triticum tauschii*, var strangulata, accession number CPI 100799. Of all the accessions of *T. tauschii* surveyed, the genome of CPI 100799 is the most closely related to the D genome of hexaploid wheat (Dr E. Lagudah, CSIRO Plant Industry, personal communication).

Triticum tauschii, var strangulata (CPI accession number 110799) was kindly provided by Dr E Lagudah. Leaves were isolated from plants grown in the glasshouse.

DNA was extracted from leaves of *Triticum tauschii* using published methods (Lagudah *et al*, 1991), partially digested with *Sau*3A, size fractionated and ligated to the arms of lambda GEM 12 (Promega). The ligated products were used to transfect the methylation tolerant strain PMC 103 (Doherty *et al*. 1993). A total of 2×10^6 primary plaques were obtained with an average insert size of about 15 kb. Thus the library contains approximately 6 genomes worth of *T. tauschii* DNA. The library was amplified and stored at 4°C until required.

Positive plaques in the genomic library were selected as those hybridising with the 5' end of a maize starch branching enzyme I cDNA (Baba *et al*, 1991) using moderately stringent conditions as described in Rahman *et al*, (1997).

Preparation of Total RNA from Wheat

Total RNA was isolated from leaves, pre-anthesis pericarp and different developmental stages of wheat

endosperm of the cultivar, Hartog and Rosella. This material was collected from both the glasshouse and the field. The method used for RNA isolation was essentially the same as that described by Higgins *et al* (1976). RNA was then quantified by UV absorption and by separation in 1.4% agarose-formaldehyde gels which were then visualized under UV light after staining with ethidium bromide (Sambrook *et al*, 1989).

DNA and RNA analysis

DNA was isolated and analysed using established protocols (Sambrook *et al*, 1989). DNA was extracted from wheat (cv. Chinese Spring) using published methods (Lagudah *et al*, 1991). Southern analysis was performed essentially as described by Jolly *et al* (1996). Briefly, 20 µg wheat DNA was digested, electrophoresed and transferred to a nylon membrane. Hybridisation was conducted at 42°C in 25% or 50% formamide, 2 x SSC, 6% Dextran Sulphate for 16h and the membrane was washed at 60°C in 2 x SSC for 3 x 1h unless otherwise indicated. Hybridisation was detected by autoradiography using Fuji X-Omat film.

RNA analysis was performed as follows. 10 µg of total RNA was separated in a 1.4% agarose-formaldehyde gel and transferred to a nylon Hybond N^+ membrane (Sambrook *et al*, 1989), and hybridized with cDNA probe at 42°C in Khandjian hybridizing buffer (Khandjian, 1989). The 3' part of wheat SBE I cDNA (designated wSBE I-D43, see Table 1) was labelled with the Rapid Multiprime DNA Probe Labelling Kit (Amersham) and used as probe. After washing at 60°C with 2 x SSC, 0.1% SDS three times, each time for about 1 to 2 hours, the membrane was visualized by overnight exposure at -80°C with X-ray film, Kodak MR.

Example 2 Frequency of Recovery of SBE I Type Clones from the Genomic Library

An estimated 2 x 10^6 plaques from the amplified library were screened using an *Eco*RI fragment that

contained 1200 bp at the 5′ end of maize SBE I (Baba *et al*, 1991) and twelve independent isolates were recovered and purified. This corresponds to the screening of somewhat fewer than the 2 x 10^6 primary plaques that exist in the original library (each of which has an average insert size of 15 kb) (Maniatis *et al*, 1982), because the amplification may lead to the representation of some sequences more than others. Assuming that the amplified library contains approximately three genomes of *T. tauschii*, the frequency with which SBE I-positive clones were recovered suggests the existence of about 5 copies of SBE I type genes within the *T. tauschii* genome.

Digestion of DNA from the twelve independent isolates by the restriction endonuclease *Bam*HI followed by hybridisation with a maize SBE I clone, suggested that the genomic clones could be separated into two broad classes (Figure 1). One class had 10 members and a representative from this class is the clone λE1 (Figure 1, lane 1); λE6 (Figure 1, lane 3) is a member of this class, but is missing the 5' end of the E1-SBE I gene because the SBE I gene is at the extremity of the cloned DNA. Further hybridisation studies at high stringency with the extreme 5' and 3' regions of the SBE I gene contained in λE1 suggested that the other clones contained either identical or very closely related genes.

The second family had two members, and of these clone λE7 (Figure 1, lane 4) was arbitrarily selected for further study. These two members did not hybridise to probes from the extreme 5' and 3' regions of the SBE I gene that were contained in λE1, indicating that they were a distinct sub-class.

The DNA from *T. tauschii* and the lambda clones λE1 and λE7 was digested with *Bam*HI and hybridised with fragment E1.1, as shown in Figure 2. This fragment contains sequences that are highly conserved (85% sequence identity over 0.3 kB between λE1 and λE7), corresponding to exons 3, 4 and 5 of the rice gene. The bands in the

genomic DNA at 0.8 kb and 1.0 kb correspond to identical sized fragments from λE1 and λE7, as shown in Figure 2; these are fragments E1.1 and E7.8 of λE1 and λE7 genomic clones respectively. Thus the arrangement of genes in the genomic clones is unlikely to be an artefact of the cloning procedure. There are also bands in the genomic DNA of approximately 2.5 kb, 4.8 kb and 8 kb in size which are not found from the digestion of λE1 or λE7; these could represent genes such as the 5' sequences of wSBE I-D1 or wSBE I-D3; see below.

Example 3 Tandem Arrangement of SBE I Type Genes in the *T. tauschii* Genome

Basic restriction endonuclease maps for λE1 and λE7 are shown in Figure 3. The map was constructed by performing a series of hybridisations of *Eco*RI or *Bam*HI digested DNA from λE1 or λE7. The probes used were the fragments generated from *Bam*HI digestion of the relevant clone. Confirmation of the maps was obtained by PCR analysis, using primers both within the insert and also from the arms of lambda itself. PCR was performed in 10 µl volume using reagents supplied by Perkin-Elmer. The primers were used at a concentration of 20 µM. The program used was 94°C, 2 min, 1 cycle, then 94°C, 30 sec; 55°C, 30 sec; 72°C, 1min for 36 cycles and then 72°C, 5 min; 25°C, 1 min.

Sequencing was performed on an ABI sequencer using the manufacturer's recommended protocols for both dye primer and dye terminator technologies. Deletions were carried out using the Erase-a-base kit from Promega.

Sequence analysis was carried out using the GCG version 7 package of computer programs (Devereaux *et al*, 1984).

The PCR products were also used as hybridisation probes. The positioning of the genes was derived from sequencing the ends of the *Bam*HI subclones and also from sequencing PCR products generated from primers based on the

insert and the lambda arms. The results indicate that there is only a single copy of a SBE I type gene within λE1. However, it is clear that λE7 resulted from the cloning of a DNA fragment from within a tandem array of the SBE I type genes. Of the three genes in the clone, which are named as wSBE I-D1, wSBE I-D2 and wSBE I-D3); only the central one (wSBE I-D2) is complete.

Example 4 Construction and Screening of cDNA Library

A wheat cDNA library was constructed from the cultivar Rosella using pooled RNA from endosperm at 8, 12, 18 and 20 days after anthesis.

The cDNA library was prepared from poly A^+ RNA that was extracted from developing wheat grains (cv. Rosella, a hexaploid soft wheat cultivar) at 8, 12, 15, 18, 21 and 30 days after anthesis. The RNA was pooled and used to synthesise cDNA that was propagated in lambda ZapII (Stratagene).

The library was screened with a genomic fragment from λE7 encompassing exons 3, 4 and 5 (fragment E7.8 in Figure 3). A number of clones were isolated. Of these an apparently full-length clone appeared to encode a novel type of cDNA for SBE I. This cDNA has been termed SBE I-D2 type cDNA. The putative protein product is compared with the maize SBE I and rice SBE I type sequences in Figure 4. The main difference is that this putative protein product is shorter at the C-terminal end, with an estimated molecular size of approximately 74 kD compared with 85 kDa for rice SBE I (Kawasaki *et al*, 1993). Note that amino acids corresponding to exon 9 of rice are missing in SBE I-D2 type cDNA, but those corresponding to exon 10 are present. There are no amino acid residues corresponding to exons 11-14 of rice; furthermore, the sequence corresponding to the last 57 amino acids of SBE I-D2 type has no significant homology to the sequence of the rice gene.

We expressed SBE I-D2 type cDNA in *E. coli* in order to examine its function. The cDNA was expressed as a fusion protein with 22 N-terminal residues of β-galactosidase and two threonine residues followed by the SBE I-D2 cDNA sequence either in or out of frame. Although an expected product of about 75 kDa in size was produced from only the in-frame fusion, we could not detect any enzyme activity from crude extracts of *E. coli* protein (data not shown). Furthermore the in-frame construct could not complement an *E. coli* strain with a defined deletion in glycogen branching, although other putative branching enzyme cDNAs have been shown to be functional by this assay (data not shown). It is therefore unclear whether the wSBE I-D2 gene in λE7 codes for an active enzyme *in vivo*.

Example 5 Gene Structure in E7

i. Sequence of wSBE I-D2

We sequenced 9.2 kb of DNA that contained wSBE I-D2. This corresponds to fragments 7.31, 7.8 and 7.18. Fragment 7.31 was sequenced in its entirety (4.1 kb), but the sequence of about 30 bases about 2 kb upstream of the start of the gene could not be obtained because it was composed entirely of Gs. Elevation of the temperature of sequencing did not overcome this problem. Fragments 7.8 (1 kb) and 7.18 (4 kb) were completely sequenced, and corresponded to 2 kb downstream of the last exon detected for this gene. It was clear that we had isolated a gene which was closely related (approximately 95% sequence identity) to the SBE I-D2 type cDNA referred to above, except that the last 200 bp at the 3' end of the cDNA are not present. The wSBE I-D2 gene includes sequences corresponding to rice exon 11 which are not in the cDNA clone. In addition it does not have exons 9, 12, 13 or 14; these are also absent from the SBE I-D2 type cDNA. The first two exons show lower identity to the corresponding exons from rice (approximately 60%) (Kawasaki *et al*, 1993) than to the other exons (about 80%). A

diagrammatic exon-intron structure of the wSBE I-D2 gene is indicated in Figure 6. The restriction map was confirmed by sequencing the PCR products that spanned fragments 7.18 and 7.8 and 7.8 and E7.31 (see Figure 3) respectively.

ii. Sequence of wSBE I-D3

This gene was not sequenced in detail, as the genomic clone did not extend far enough to include the 5' end of the sequence. The sequence is of a SBE-I type. The orientation of the gene is evident from sequencing of the relevant *Bam*HI fragments, and was confirmed by sequence analysis of a PCR product generated using primers from the right arm of lambda and a primer from the middle of the gene. The sequence homology with wSBEI-D2 is about 80% over the regions examined. The 2 kb sequenced corresponded to exons 5 and 6 of the rice gene; these sequences were obtained by sequencing the ends of fragments 7.5, 7.4 and 7.14 respectively, although the sequences from the left end of fragment 7.14 did not show any homology to the rice sequences. The gene does not appear to share the 3' end of SBE I-D2 type cDNA, as a probe from 500 bp at the 3' end of the cDNA (including sequences corresponding to exons 8 and 10 from rice) did not hybridise to fragment 7.14, although it hybridised to fragment 7.18 (data not shown).

iii. Sequence of wSBE I-D1

This gene was also not sequenced in detail, as it was clear that the genomic clone did not extend far enough to include the 5' sequences. Limited sequencing suggests that it is also a SBE I type gene. The orientation relative to the left arm of lambda was confirmed by sequencing a PCR product that used a primer from the left arm of lambda and one from the middle of the gene (as above). Its sequence homology with wSBE I-D2 ,D3 and D4 (see below) is about 75% in the region sequenced corresponding to a part of exon 4 of the rice gene.

Starch branching enzymes are members of the α-amylase protein family, and in a recent survey Svensson (1994) identified eight residues in this family that are invariant, seven in the catalytic site and a glycine in a short turn. Of the seven catalytic residues, four are changed in SBE I-D2 type. However, additional variation in the 'conserved' residues may come to light when more plant cDNAs for branching enzyme I are available for analysis. In addition, although exons 9, 11, 12, 13 and 14 from rice are not present in the SBE I-D2 type cDNA (Figure 5), comparison of the maize and rice SBE I sequences indicate that the 3' region (from amino acid residue 730 of maize) is much more variable than the 5' and central regions (Figure 4). The active sites of rice and maize SBE I sequences, as indicated by Svensson (1994), are encoded by sequences that are in the central portion of the gene. When SBE II sequences from *Arabidopsis* were compared by Fisher *et al* (1996) they also found variation at the 3' and 5' ends. SBE I-D2 type cDNA may encode a novel type of branching enzyme whose activity is not adequately detected in the current assays for detecting branching enzyme activity; alternatively the cDNA may correspond to an endosperm mRNA that does not produce a functional protein.

Example 6 Cloning of Specific cDNA Regions of Wheat SBEI Using RT-PCR

The first strand cDNAs were synthesized from 1 μg of total RNA, derived from endosperm 12 days after pollination, as described by Sambrook *et al* (1989), and then used as templates to amplify two specific cDNA regions of wheat SBE I by PCR.

Two pairs of primers were used to obtain the cDNA clones BED1 and BED3 (Table 1). Primers used for cloning of BED3 were the degenerate primer NTS5'

5' GGC NAC NGC NGA G/AGA C/TGG 3' (SEQ ID NO. 1)

in which the 5' end is at position 168 of wSBE I-D4 cDNA, as shown in Table 1), based on the N-terminal sequence of wheat SBE I, and NTS3'

5' TAC ATT TCC TTG TCC ATCA 3' (SEQ ID NO. 2)

in which the 5' end is at position 1590 of wSBE I-D4 cDNA, (see Table 1), derived from the conserved regions of the nucleotide sequences of BED5 and the maize and rice SBE I cDNAs. For clone BED1, the primers used were BEC5'

5' ATC ACG AGA GCT TGC TCA (SEQ ID NO. 3)

in which the 5' end is at position 1 of wSBE I-D4 cDNA, (see Table 1); the sequence was based on wSBE I-D4, and BEC3'

5' CGG TAC ACA GTT GCG TCA TTT TC 3' (SEQ ID NO. 4)

in which the 5'end is at position 334 of wSBE I-D4 cDNA (see Table 1), and the sequence was based on BED 3.

Table 1

Positions of Sequences Relative to wSBE I-D4 Sequences

Sequence Name	wSBE I-D4 Sequence	wSBE I-D4 cDNA Sequenc
Putative initiation of translation	4900	11
N-terminal sequence of SBE I	5550	124
End of translated SBE I sequence	10225	2431
End of wSBE I-D4 cDNA sequence	10461	2687
wSBE I-D45	4870, 5860	1,357
wSBE I-D43	9430, 10435	see below
wSBE-I-D43C	see above	2338,2657
wSBE I-D4R	2kb of sequence approx 600bp 3' to position 10461	not applicable
E 1.1	5680, 6400	380,630
BED 1	not referred to	1,354
BED 2	not referred to	169,418
BED 3	not referred to	151,1601
BED 4	not referred to	867,2372
BED 5	not referred to	867,2687

Example 7 Identification of a gene encoding the major N-terminal of SBE I from the endosperm

We have isolated two classes of SBE I genomic clones from *T. tauschii*. One class contained two genomic clone isolates, and this class has been characterised in some detail (Rahman *et al*, 1997). The complete gene contained within this class of clones was termed *wSBE I-D2*; there were additional genes at either ends of the clone, and these were designated *wSBE I-D1* and *wSBE I-D3*. The other class contained nine genomic clone isolates. Of these λE1 was arbitrarily taken as a representative clone, and its restriction map is shown in Figure 3; the SBE I gene contained in this clone was called *wSBE I-D4*. Fragments E1.1 (0.8 kb) and E1.2 (2.1 kb) and fragments E1.7 (4.8 kb) and E1.5 (3 kb) respectively were completely sequenced. Fragment E1.7 was found to encode the N-terminal of the SBE I, which is found in the endosperm as described in Morell *et al* (1997). This is shown in Figure 4. Using antibodies raised against the N-terminal sequence, Morell *et al* (1997) found that the D genome isoform was the most highly expressed in the cultivars Rosella and Chinese Spring. We have thus isolated from *T. tauschii* a gene, *wSBE I-D4*, whose homologue in the hexaploid wheat genome encodes the major isoform for SBE I that is found in the wheat endosperm.

All nine genomic clones isolated from *T. tauschii* appear to contain the *wSBE I-D4* gene, or very similar genes, on the basis of PCR amplification and hybridisation experiments. However, the restriction patterns obtained for the clones differ with *Bam*HI and *Eco*RI, among other enzymes, indicating that either the clones represent near-identical but distinct genes or they represent the same gene isolated in distinct products of the *Sau* 3A digest used to generate the library.

Example 8 Investigation of other SBE I genomic clones isolated

All ten members of the E1-like class of SBE I genomic clones were investigated by hybridisation with probes derived from fragment E1.7 (sequence *wSBE I-D45*, encoding the translation start signal and the first 100 amino acids from the N-terminal end and intron sequences; see Table 2) and from fragment E1.5 (sequence *wSBE I-D43*, corresponding largely to the 3' untranslated sequence and containing intron sequences, see Table 1). The results obtained were consistent with one type of gene being isolated in different fragments in the different clones, as shown in Figure 5. The PCR products were obtained from the clones λE1, 2, 9, 14, 27, 31 and 52. These hybridised to *wSBE I-D45* using primers that amplify near the 5' end of the gene (positions 5590-6162 of *wSBE I-D4)*. Sequencing showed no differences in sequence of a 200 bp product.

Analysis of the promoter for *wSBE I-D4* allows us to investigate the presence of motifs previously described for promoters that regulate gene expression in the endosperm. Kreis *et al* (1985) compared prolamin promoters, and suggested that the presence of a motif approximately -300 bp upstream of the transcription start point, called the endosperm box, was responsible for endosperm-specific expression. The endosperm box was subsequently considered to consist of two different motifs: the endosperm motif (EM) (canonical sequence TGTAAAG) and the GCN 4 motif (canonical sequence G/ATGAG/CTCAT). The GCN4 box is considered to regulate expression according to nitrogen availability (Muller and Knudsen, 1993). The *wSBE I-D4* promoter contains a number of imperfect EM-like motifs at approximately -100,-300 and -400 as well as further upstream. However, no GCN4 motifs could be found, which lends support to the idea that this motif regulates response to nitrogen, as starch biosynthesis is not as directly dependent on the nitrogen status of the plant as

storage protein synthesis. Comparison of the promoters for *wSBE I-D4* and *D2* (Rahman *et al*, 1997) indicates that although there are no extensive sequence homologies there is a region of about 100 bp immediately before the first encoded methionine where the homology is 61% between the two promoters. In particular there is an almost perfect match in the sequence over twenty base pairs CTCGTTGCTTCC/TACTCCACT, (position 4723-4742 of the *wSBE I* sequence), but the significance of this is hard to gauge, as it does not occur in the rice promoter for SBE I. The availability of more promoters for starch biosynthetic enzymes may allow firmer conclusions to be drawn. There are putative CAAT and TATA motifs at positions 4870 and 4830 respectively of *wSBE I-D4* sequence. The putative start of translation of the mRNA is at position 4900 of *wSBE I-D4*.

Figure 6 shows the structure of the *wSBE I-D4* gene, compared with the genes from rice and wheat (Kawasaki *et al*, 1993; Rahman *et al*, 1997). The rice *SBE I* has 14 exons compared with 13 for *wSBE I-D4* and 10 for *wSBE I-D2*. There is good conservation of exon-intron structure between the three genes, except at the extreme 5' end. In particular the sizes of intron 1 and intron 2 are very different between rice *SBE I* and *wSBE I-D4*.

Example 9 Isolation of cDNA for SBE I

Using the maize starch branching enzyme I cDNA as a probe (Baba *et al*, 1991), 10 positive plaques were recovered by screening approximately 10^5 plaques from a wheat endosperm cDNA library. On purifying and sequencing these plaques it was clear that even the longest clone (BED5, 1822 bp) did not encode the N-terminal sequence obtained from protein analysis. Degenerate primers based on the protein N-terminal and the sequence from BED5 were then used to amplify the 5' region: this produced a cDNA clone termed BED 3 (Table 1 and Figure 7). This cDNA clone overlapped extensively and had 100% sequence identity with

BED5 and BED4 (Figure 7). As almost the entire protein N-terminal sequence had been included in the primer sequence design, this did not provide independent evidence of the selection of a cDNA sequence in the endosperm that encoded the protein sequence of the main form of SBE I. Using a BED3 to screen a second cDNA library produced BED2, which is shorter than BED3 but confirmed the BED3 sequence at 100% identity between positions 169 and 418 (Figure 7 and Table 1). In addition the entire cDNA sequence for BED3 could be detected at a 100% match in the genomic clone λE1. Primers based on the putative transcription start point combined with a primer based on the incomplete cDNAs recovered were then used to obtain a PCR product from total endosperm RNA by reverse transcription. This led to the isolation of the cDNA clone, BED1, of 300 bp, whose sequence is shown in Figure 7. By analysing this product, a sequence was again obtained that could be found exactly in the genomic clone λE1, and which overlapped precisely with BED3.

The N-terminal of the protein matches that of SBE I isolated from wheat endosperm by Morell *et al* (1997), and thus it is likely that *wSBE I-D4* cDNA represents the gene for the predominant SBE I isoform expressed in the endosperm. The encoded protein is 87 kDa; this is similar to proteins encoded by maize (Baba *et al*, 1991) and rice (Nakamura *et al*, 1992) cDNAs for SBE I and is distinct from the *wSBE I-D2* cDNA described previously, in which the encoded protein was 74 kDa (Rahman *et al*, 1997).

Five cDNA clones were sequenced and their sequences were assembled into one contiguous sequence using a GCG program (Devereaux *et al*, 1984). This is illustrated in Figure 8. The intact cDNA sequence, *wSBE I-D4* cDNA, is 2687 bp and contains one large open reading frame (ORF), which starts at nucleotides 11 to 13 and ends at nucleotides 2432 to 2434. It encodes a polypeptide of 807 amino acids with a molecular weight of 87 kDa. Comparison of the amino acid sequence encoded by *wSBE I-D4*

cDNA with that encoded by maize and rice *SBE I* cDNAs showed that there is 75-80% identity between any of two these sequences at the nucleotide level and almost 90% at the amino acid level. Alignment of these three polypeptide sequences, as shown in Figure 9, along with the deduced sequences for pea, potato and *wSBE I-D2* type cDNA, indicated that the sequences in the central region are highly conserved, and sequences at the 5' end (about 80 amino acids) and the 3' end (about 60 amino acids) are variable.

Svensson *et al* (1994) indicated that there were several invariant residues in sequences of the α-amylase super-family of proteins to which SBE I belongs. In the sequence of maize SBE I these are in motifs commencing at amino acid residue positions 341,415,472,537 respectively; these are also encoded in the *wSBE I-D4* sequence (Figure 7), further supporting the view that this gene encodes a functional enzyme. This is in contrast to the results with the *wSBE I-D2* gene, where three of the conserved motifs appear not to be encoded (Rahman *et al*, 1997).

There is about 90% sequence identity in the deduced amino acid sequence between *wSBE I-D4* cDNA and rice *SBE I* cDNA in the central portion of the molecule (between residues 160 and 740 for the deduced amino acid product from *wSBE I-D4* cDNA). The sequence identity of the deduced amino acid sequence of the *wSBE I-D4* cDNA to the deduced amino acid sequence of *wSBE I-D2* is somewhat lower (85% for the most conserved region, between residues 285 to 390 for the deduced product of *wSBE I-D4* cDNA). Surprisingly, however, *wSBE I-D4* cDNA is missing the sequence that encodes amino acids at positions 30 to 58 in rice SBE I (see Figure 8). This corresponds to residues within the transit peptide of rice SBE I. A corresponding sequence also occurs in the deduced amino acid sequence from maize *SBE I* (Baba *et al*, 1991) and *wSBE I-D2* type cDNA (Rahman *et al*, 1997). Consequently the transit sequence

encoded by *wSBE I-D4* cDNA is unusally short, containing only 38 amino acids, compared with 55-60 amino acids deduced for most starch biosynthetic enzymes in cereals (see for example Ainsworth, 1993; Nair *et al*, 1997). The *wSBE I-D4* gene does contain this sequence, but this does not appear to be transcribed into the major species of RNA from this gene, although it can be detected at low relative abundance. This raises the possibility of alternative splicing of the *wSBE I-D4* transcript, and also the question of the relative efficiency of translation/transport of the two isoforms. The possibility of alternative splicing in both rice and wheat has been considered for soluble starch synthase (Baba *et al*, Rahman *et al*, 1995). Alternative splicing of soluble starch synthase would give a transit sequence of 40 amino acids, which is the same length proposed for the product of *wSBE I-D4* cDNA.

We have previously used probes based on exons 4, 5 and 6 (E7.8 and E1.1, see Rahman *et al.*, 1997) of *wSBE-D2* to probe wheat and *T. tauschii* genomic DNA cleaved with *Pvu*II and *Bam*HI respectively. This region is highly conserved within rice *SBE I*, *wSBE I-D2* and *wSBE I-D4* and produced ten bands with wheat DNA and five with *T. tauschii* DNA. Neither *Pvu*II nor *Bam*HI cleaved within the probe sequences suggesting that each band represented a single type of SBE I gene. We have described four SBE I genes from *T. tauschii*: *wSBE I-D1*, *wSBE I-D2*, *wSBE I-D3* and *wSBE I-D4* (Rahman *et al*, 1997 and this specification), and so we may have accounted for most of the genes in *T. tauschii* and, by extension, the genes from the D genome of wheat. In wheat, at least two hybridising bands could be assigned to each of chromosomes 7A, 7B and 7D.

Example 10 Tissue specificity and expression during endosperm development

The 300 bp of 3' untranslated sequence of *wSBE I-D4* cDNA does not show any homology with either the *wSBE I-D2* type cDNA that we have described earlier (Rahman

et al, 1997) or with BE-I from rice, as shown in Figure 7. We have called this sequence *wSBE I-D43C*. It seemed likely that *wSBE I-D43C* would be a specific probe for this class of SBE-I, and thus it was used to investigate the tissue specificity. The results are shown in Figure 10. A RNA species of about 2700 bases in size was found to hybridise. This is very close to the size of the *wSBE I-D4* cDNA sequence. RNA hybridising to wSBE-I-D43C is most abundant at the mid-stage of endosperm development (Figure 10) and in field grown material is relatively constant during the period 12-18 days, the time at which there is rapid starch and storage protein accummulation (Morell *et al*, 1995).

The sequence contained within the *wSBE I-D4* gene appears to be expressed only in the endosperm (Figure 9). We could not detect any expression in the leaf. This could be because another isoform is expressed in the leaf, and/or because the amount of SBE I present in the leaf is much less than what is required in the endosperm. Isolation of SBE I clones from a leaf cDNA library would enable this question to be resolved.

Example 11 Intron-Exon Structure of SBE I

By comparison of the cDNA sequence of SBE I with that of *wSBE I-D4* we can deduce the intron-exon structure of the gene for the major isoform of SBE I that is found in the endosperm. The structure contains 14 exons compared to 14 for rice (Kawasaki *et al*, 1993). These 14 exons are spread over 6 kb of sequence, a distance similar to that found in both rice *SBE I* and *wSBE I-D2*. A dotplot comparison of *wSBE I-D4* sequence and that of rice *SBE I* sequence, depicted in Figure 11, shows good sequence identity over almost the entire gene starting from about position 5100 of *wSBE I-D4*; the identity is poor over the first 5 kb of sequence corresponding largely to the promoter sequences. The sequence identity over introns (about 60%) is lower than over exons (about 85%).

Example 12 Repeated Sequences in SBE I

Sequencing of *wSBE I-D4* revealed there was a repeated sequence of at least 300 bp contained in a 2kb fragment about 600 bp after the 3' end of the gene. We have called this sequence *wSBE I-D4R*. This repeated sequence is within fragment E1.5 (Figure 10 and Table 2) and is flanked by non-repetitive sequences from the genomic clone. We have previously shown that the restriction pattern obtained by digesting λE1 with the restriction enzyme *Bam*HI is also obtained when *T. tauschii* DNA is digested. Thus *wSBE I-D4R* is unlikely to be a cloning artefact. A search of the GeneBank Database searches revealed that *wSBE I-D4R* shared no significant homology with any sequence in the database. Hybridisation experiments with *wSBE I-D4R* showed that all of the other *SBE I-D4* type genomic clones (except number 29) contained this repeated sequence (data not shown). The *wSBE I-D4R* sequence was not highly repeated and occurred in the wheat genome with a similar frequency as the w*SBE I-D4* sequence.

When *SBE I-D4R* was used as the probe on wheat DNA from the nulli-tetra lines, four bands were obtained; two of these bands could be assigned to chromosome 7A and the others to chromosomes 7B and 7D (Figure 11C). One of the two *Bam*HI fragments from wheat DNA which could be assigned to chromosome 7A was distinct from the single band from chromosome 7A detected using *wSBE I-D43* as the probe; the other three bands coincided in the autoradiograph with bands obtained with *wSBE I-D43*, and are likely to represent the same fragment. However, one of these fragments was distinct from the *Bam*HI fragment that hybridised to the *wSBE I-D43* sequence. In *wSBE I-D4* the *wSBE I-D43* sequence is only 300 bp upstream of *wSBE I-D4R*, and occurs in the same *Bam*HI fragment. These results suggest that the *wSBE I-D4R* sequence can occur independently of *wSBE I-D4* in the wheat genome.

Example 13 Isolation of Genomic Clones Encoding SBE II

Screening of a cDNA library prepared from the wheat endosperm with the maize BE I clone (Baba *et al*, 1991) at low stringency led to the isolation of two classes of positive plaques. One class was strongly hybridising, and led to the isolation of wheat SBE I cDNA clones, as described in Example 5 and in Rahman *et al* (1997). The second class was weakly hybridising, and one member of this class was purified. This weakly hybridising clone was termed SBE-9, and on sequencing was found to contain a sequence that was distinct from that for SBE I. This sequence showed greatest homology to maize BE II sequences, and was considered to encode part of the wheat SBE II sequence.

The screening of approximately 5 x 10^5 plaques from a genomic library constructed from *T. tauschii* with the SBE-9 sequence led to the isolation of four plaques that were positive. These were designated *wSBE II*-D1 to *wSBE II-D4* respectively, and were purified and analysed by restriction mapping. Although they all had different hybridization patterns with SBE-9, as shown in Figure 15, the results were consistent with the isolation of the same gene in different-sized fragments.

Example 14 Identification of the N-terminal sequence of SBE II

Sequencing of the SBE II gene contained in clone 2, termed *SBE II-D1*, showed that it coded for the N-terminal sequence of the major isoform of SBE II as identified by Morell *et al* (1997). This is shown in Figure 16a.

Example 15 Intron-Exon Structure of the SBE II Gene

In addition to encoding the N-terminal sequence of sBE II, as shown in Example 10, the cDNA sequence reported by Nair *et al* (1997) was also found to have 100% sequence identity with part of the sequence of *wSBE II-D1*.

Thus the intron-exon structure can be deduced, and this is shown in Figure 17.

Example 16 Number of SBE II Genes in *T. tauschii* and Wheat

Hybridisation of the SBE II conserved region with *T. tauschii* DNA revealed the presence of three gene classes. However, in our screening we only recovered one class. Hybridisation to wheat DNA indicated that the locus for SBE II was on chromosome 2, with approximately 5 loci in wheat; most of these appear to be on chromosome 2D, as shown in Figure 18.

Example 17 Expression of SBE II

Investigation of the pattern of expression of SBE II revealed that the gene was only expressed in the endosperm. However the timing of expression was quite distinct from that of SBE I, as illustrated in Figure 10.

Whereas SBE I gene expression is only clearly detectable from the mid-stage of endosperm development (Figure 7), SBE II gene expression is clearly seen much earlier, in endosperm tissue at 5-8 days after development (Figure 10), corresponding to an early stage of endosperm development in Figure 7.

Example 18 RT-PCT Amplication of SSS cDNA Sequence from Wheat

A conserved sequence region was used for the synthesis of primers for amplification of SSS by comparison with the nucleotide sequences encoding soluble starch synthases of rice and pea. A 300 bp RT-PCR product was obtained by amplification of cDNA from wheat endosperm at 12 days post anthesis. The 300 bp RT-PCT product was then cloned, and its sequence analysed. The comparison of its sequence with rice SSS cDNA showed about 80% sequence homology. The 300 bp RT-PCR product was 100% homologous to

the partial sequence of a wheat SSS in the database produced by Block *et al* (1997).

Example 19 Cloning of Wheat Soluble Starch Synthase cDNA

The 300 bp cDNA fragment of wheat soluble starch synthase isolated in Example 14 was used as a probe for the screening of a wheat endosperm cDNA library (Rahman *et al*, 1996). Eight cDNA clones were selected. One of the largest cDNA clones was used for DNA sequencing analysis, and gave a 2662 bp nucleotide sequence. A large open reading frame of this cDNA encoded a 647 amino acid polypeptide, starting at nucleotides 247 to 250 and terminating at nucleotides 2198 to 2200. The deduced polypeptide was shown by protein sequence analysis to contain the N-terminal sequence of a 75 kDa granule-bound protein (Rahman *et al*, 1995). This is illustrated in Figures 21a and 21b. The location of the 75 kDa protein was determined both the soluble fraction and starch granule-bound fraction by the method of Denyer *et al* (1995). Thus this cDNA clone encoded a polypeptide comprising a 41 amino acid transit peptide and a 606 amino acid mature peptide. The cleavage site LRRL was located at amino acids 36 to 39 of the transit peptide of this deduced polypeptide.

Comparison of wheat SSS with rice SSS and potato SSS showed that there is 87.4% or 75.9% homology at the amino acid level and 74.7% or 58.1% homology at the nucleotide level. Some amino acids in the at N-terminal sequences of the SSS of wheat and rice were conserved.

Example 20 Isolation of Genomic Clone of Wheat Soluble Starch Synthase

Seven genomic clones were obtained with a 300 bp cDNA probe by screening approximately 5 x 10^5 plaques from a genomic DNA library of *Triticum tauschii*, as described above. DNA was purified from 5 of these clones and

digested with *Bam*HI and *Sac*I. Southern hybridization analysis using the 300 bp cDNA as probe showed that these clones could be classified into two classes, as shown in Figure 23. One genomic clone, sg3, contained a long insert, and was digested with *Bam*HI or *Sac*I and subcloned into pBluescript KS+ vector.

These subclones were analysed by sequencing, and the sequence of the genomic clone sg3is shown in Figure 21c

Example 21 Northern Hybridization Analysis of the Expression of Genes Encoding Soluble Starch Synthase

Total RNAs were purified from leaves, pre-anthesis material, and various stages of developmental endosperm at 5-8, 10-15 and 18-22 days post anthesis. Northern hybridization analysis showed that mRNAs encoding wheat SSS were specifically expressed in developmental endosperm. Expression of this mRNAs in the leaves and pre-anthesis materials could not be detected by northern hybridization analysis under this experimental condition. Wheat SSS mRNAs started to express at high levels at an early stage of endosperm, 5-8 days post anthesis, and the expression level in endosperm at 10-15 days post anthesis, was reduced. These results are summarized in Figure 24.

Example 22 Genomic Localisation of Wheat Soluble Starch Synthase

DNA from chromosome engineered lines was digested with the restriction enzyme BamHI and blotted onto supported nitrocellulose membranes. A probe prepared from the 3' end of the cDNA sequence, from positions 2345 to 2548, was used to hybridise to this DNA. The presence of a specific band was shown to be associated with the presence of chromosomes 7A (Figure 25). These data demonstrate location of the SSS gene on chromosome 7.

Example 23 Isolation of SSS Promoter

We have isolated the promoter that drives this pattern of expression for SSS. The pattern of expression for SSS is very similar to that for SBE II: the SSS gene transcript is detectable from an early stage of endosperm development until the endosperm matures. The sequence of this promoter is given in Figure 26.

Example 24 Isolation of the Gene Encoding Debranching Enzyme from Wheat

The *sugary* mutation in maize results in mature dried kernels that have a glassy and translucent appearance; immature mature kernels accumulate sucrose and other simple sugars, as well as the water-soluble polysaccharide phytoglycogen (Black *et al*, 1966). Most data indicates that in *sugary* mutants the concentration of amylose is increased relative to that of amylopection. Analysis of a particular *sugary* mutation (*su-Ref*) by James *et al*, (1995) led to the isolation of a cDNA that shared significant sequence identity with bacterial enzymes that hydrolyse the α 1,6-glucosyl linkages of starch, such as an isoamylase from *Pseudomonas* (Amemura *et al*, 1988), *ie*. bacterial debranching enzymes.

We have now isolated a sequence amplified from wheat endosperm cDNA using the polymerase chain reaction (PCR). This sequence is highly homologous to the sequence for the *sugary* gene isolated by James *et al*, (1995). This sequence has been used to isolate homologous cDNA sequences from a wheat endosperm library and genomic sequences from *Triticum tauschii*.

Comparison of the deduced amino acid sequences of DBE from maize with spinach (Accession SOPULSO, unpublished), *Pseudomonas* (Amemura *et al*, 1988) and rice (Nakamura *et al*, 1997) enabled us to deduce sequences which could be useful in wheat. When these sequences were used as PCR amplification primers with wheat genomic DNA a product of 256 bp was produced. This was sequenced and was

compared to the sequence of maize *sugary* isolated by James *et al*, (1995). The results are shown in Figure 28a and 28b. This sequence has been termed wheat debranching enzyme sequence I (WDBE-I).

WDBE-1 was used to investigate a cDNA library constructed from wheat endosperm (Rahman *et al*, 1997) enables us to isolate two cDNA clones which hybridise strongly to the WDBE-I probe. Use of WDBE 1 to investigate a genomic library constructed from *T. tauschii*, as described above has led to the isolation of four genomic clones which hybridised strongly to the WDBE-I sequence. Hybridization of WDBE-I to DNA from *T. tauschii* indicates one hybridizing fragment (Figure 29).

We have clearly isolated a sequence from the wheat genome that has high identity to the debranching enzyme cDNA of maize characterised by James *et al* (1997). The isolation of homologous cDNA sequences and genomic sequences enables further characterisation of the debranching enzyme cDNA and promoter sequences from wheat and *T. tauschii*. These sequences and the WDBE I sequences shown herein are useful in the manipulation of wheat starch structure through genetic manipulation and in the screening for mutants at the equivalent *sugary* locus in wheat.

Example 25 Use of probes from granule-bound starch synthase and SBE II sequences to identify null or altered alleles for use in breeding programmes

There are two general strategies for obtaining wheats with altered starch structure:

(a) using genetic engineering strategies to suppress the activity of a specific gene, or to introduce a novel gene into a wheat line.

(b) selecting among existing variation in wheat for missing ("null") or altered alleles of a gene in each of the genomes of wheat, and combining these by plant breeding.

DNA primer sets were designed to enable amplification of the first 9 introns of the SBE II gene using PCR. The design of the primer sets is illustrated in Figure 30. Primers were based on the wSBE II-D1 sequence (Figure 19) and were designed such that intron sequences in the wSBE II sequence (deduced from Figure 17) were amplified by PCR. These primer sets individually amplify the first 9 introns of SBE II. One primer set, for intron 6, was found to amplify products from each of chromosomes 2A, 2B and 2D of wheat. This is shown in Figure 31, which illustrates results obtained with chromosome 2 nullisomic tetrasomic lines of the cultivar Chinese Spring.

Figure 32 compares results of amplification with the Intron 6 primer set for normal lines of the cultivars Chinese Spring and Rosella. In Chinese Spring a PCR product of 213 bp is absent, indicating that this cultivar possesses a potential null allele. Thus Chinese Spring can be used as a parental line for breeding programmes for generation of new lines in which expression of SBE II is diminished or abolished, with consequent increase in amylose content of the wheat grain. Thus a high amylose wheat can be produced.

It will be apparent to the person skilled in the art that while the invention has been described in some detail for the purposes of clarity and understanding, various modifications and alterations to the embodiments and methods described herein may be made without departing from the scope of the inventive concept disclosed in this specification.

Reference cited herein are listed on the following pages, and are incorporated herein by this reference.

REFERENCES

Ainsworth, C., Clark, J. and Balsdon, J.
Plant Molecular Biology, 1993 22 67-82

Amemura, A., Chakrabort, R., Fujita, M., Noumi, T. and Futai, M.
J. Biol. Chem., 1988 263 9271-9275

Baba, T., Kimura, K., Mizuno, K., Etoh, H., Ishida,Y., Shida, O. and Arai, Y.
Biochem. Biophys. Res. Commun., 1991 181 87-94.

Black, R.C., Loerch, J.D., McARdle, F.J. and Creech, R.G.
Genetics, 1966 53 661-668

Burton, R.A., Bewley, J.D., Smith, A.M., Bhattacharya, M.K., Tatge, H., Ring,S., Bull, V., Hamilton, W.D.O. and Martin, C.
The Plant Journal, 1995 7 3-15.

Cangiano, G., La Volpe, A., Poulsen, P. and Kreiberg, J.D.
Plant Physiology, 1993 102 1053-1054.

Clarke, B.C., Mukai, Y. and Appels, R.
Chromosoma, 1996 105 269-275

Devereaux, J., Haeberli, P. and Smithies, O.
Nucleic Acids Res., 1984 12, 387-395.

Denyer, K., Hylton, C.M., Jenner, C.F. and Smith, A.M.
Planta, 1995 196 256-265

Doherty, J.P., Lindeman, R., Trent, R.J., Graham, M.W. and Woodcock, D.M.
Gene, 1992 124 113-120

Gill, B.S. and Appels, R.
Plant Syst. Evol., 1988 160 77-90.

Jahne, A., Lazzeri, P.A., Jager-Gussen, M. and Lorz, H.
Theor. Appl. Genet., 1991 82 47-80

James, M.G., Robertson, D.S. and Myers, A.M.
Plant Cell, 1995 7 417-429

Jolly, C.J., Glenn, G.M. and Rahman, S.
Proc. Natl Acad. Sci., 1996 93 2408-2413.

Kawasaki, T., Mizuno, K., Baba, T. and Shimada, H.
Molec. Gen. Genet., 1993 237 10-16.

Lagudah, E.S., Appels, R. and McNeill, D.
Genome, 1991 34 387-395

Lazzeri, P.A., Brettschneider, R., Luhrs, R. and Lorz, H.
Theor. Appl. Genet., 1991 81 437-444

Maniatis, T., Fritsch, E.F. and Sambrook, J.
Molecular cloning. A Laboratory Manual., New York. Cold Spring Harbor Laboratory, 1982

Mizuno, K., Kawasaki, T., Shimada, H., Satoh, H., Koyabashi, E., Okumura, S., Arai, Y. and Baba, T.
J.Biol. Chem., 1993 268 19084-19091.

Martin, C. and Smith, A.
The Plant Cell, 1995 7 971-985.

Morell, M.K., Blennow, A., Kosar-Hashemi, B. and Samuel, M.S.
Plant Physiol., 1996 113 201-208.

Morell, M.K., Rahman, S., Abrahams, S.L. and Appels, R.
Aust.J.of Plant Physiol., 1995 22 647-660.

Nair, R., Baga, M., Scoles, G.J., Kartha, K. and Chibbar, R.
Plant Science, 1997 1222 153-163

Nakamura, Y., Takeichi, T., Kawaguchi, K. and Yamanouchi, H.
Physiologia Plantarum, 1992 84 329-335.

Nakamura, Y., Umemoto, T. and Sasaki, T.
Planta, 1996 199 209-214

Preiss, J.
Biology and Molecular Biology of starch synthesis and its regulation. In 'Oxford Surveys of Plant Molecular and Cell Biology., 1991 Vol. 7.' (Ed. B. J. Miflin.) pp. 59-114.(Oxford University Press: Oxford.)

Rahman, S., Kosar-Hashemi, B., Samuel, M., Hill, A., Abbott, D.C., Skerritt, J.H., Preiss, J., Appels, R. and Morell, M.
Aust. J. Plant Physiol., 1995 22 793-803.

Rahman, S., Abrahams, S., Mukai, Y., Abbott, D., Samuel, M., Morell, M. and Appels, R.
Genome, 1997 40 465-474

Sambrook, J., Fritsch, E.F. and Maniatis, T.
Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory Press, 2nd ed 1989)

Svensson, B.
Plant Mol. Biol., 1994 25 141-157.

Tingay, S., McElroy, D., Kalla, R., Fieg, S., Wang, M., Thornton, S. and Bretell, R.
The Plant Journal, 1997 11 1369-1376

Wan, Y. and Lemaux, P.G.
Plant Physiology, 1994 104 37-48

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION and
THE AUSTRALIAN NATIONAL UNIVERSITY

12 September 1997

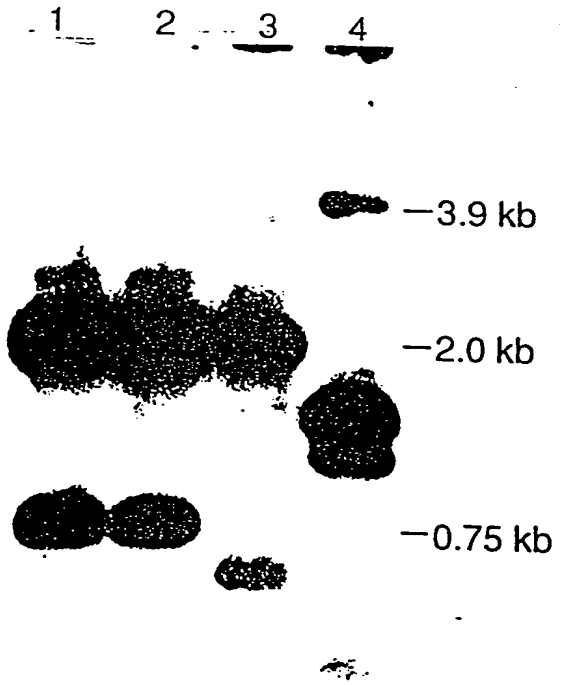


FIGURE 1

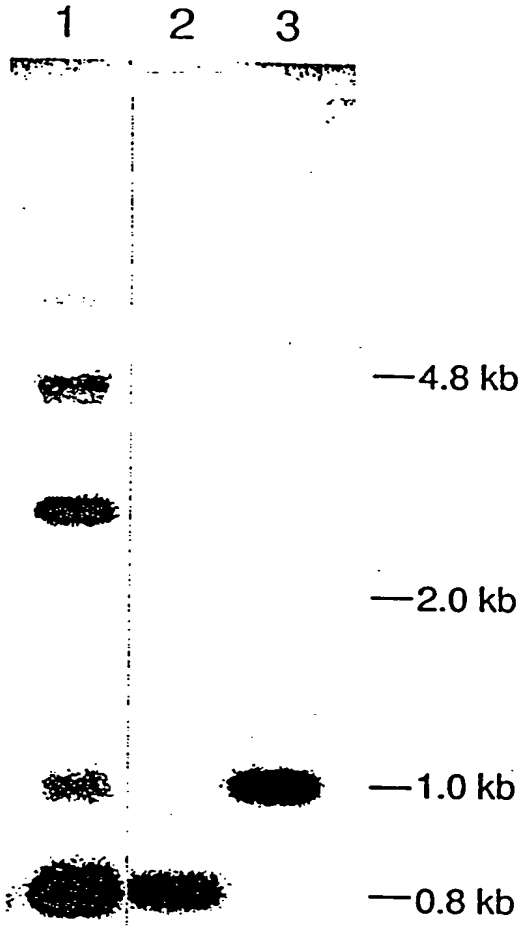
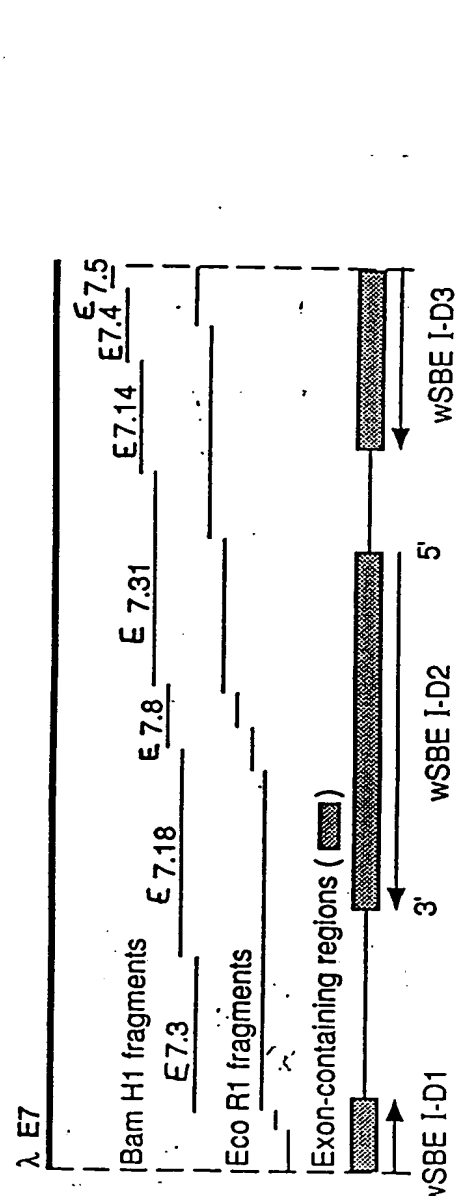


FIGURE 2


λ E7
Bam H1 fragments
E7.3
E7.18
E7.8
E7.31
E7.14
E7.4
E7.5
Eco R1 fragments
Exon-containing regions ( )
wSBE I-D1
3'
wSBE I-D2
5'
wSBE I-D3

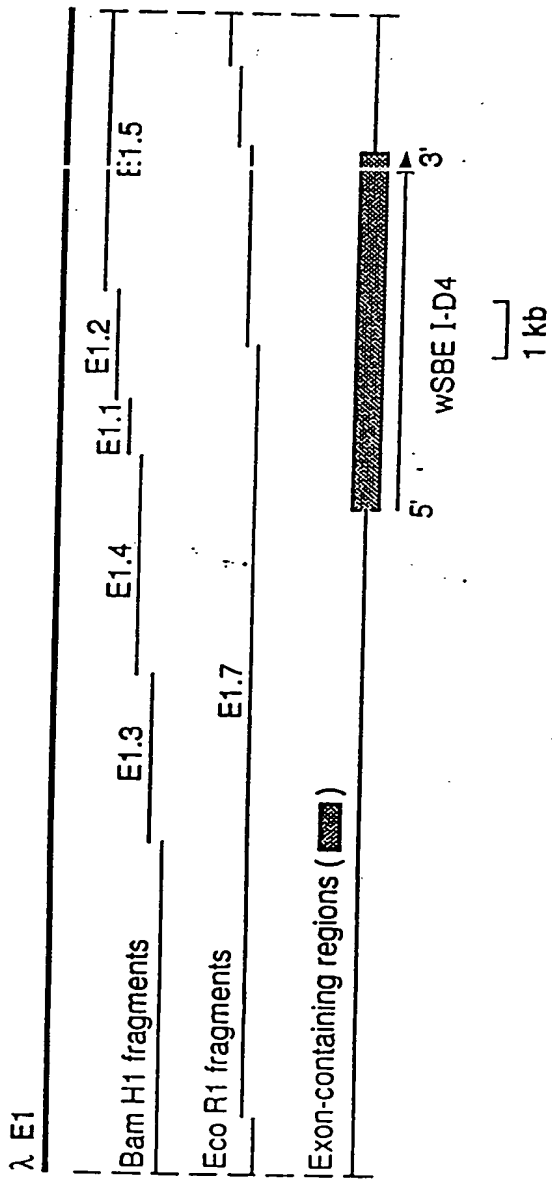

λ E1
Bam H1 fragments
E1.3
E1.4
E1.1
E1.2
E1.5
Eco R1 fragments
E1.7
Exon-containing regions ( )
5'
3'
wSBE I-D4
1 kb

FIGURE 3

DNA
5' TCCCGTGTCTGCGCCAAGAGACTACACCATGGCAACAGCTGAAGATGGTGTTGGCGACCT 5'
3' AGGGCACAGACGCGGTTCTCTGATGTGGTACCGTTGTCGACTTCTACCACAACCGCTGGA 3'
possible reading frames
S R V C A K R L H H G N S * R W C W R P
P V S A P R D Y T M A T A E D G V G D L
P C L R Q E T T P W Q Q L K M V L A T F
true N-terminal sequence for BE-1 (Morell et al, 1997)
V S A P R D Y T M A T A E D G V

FIGURE 4

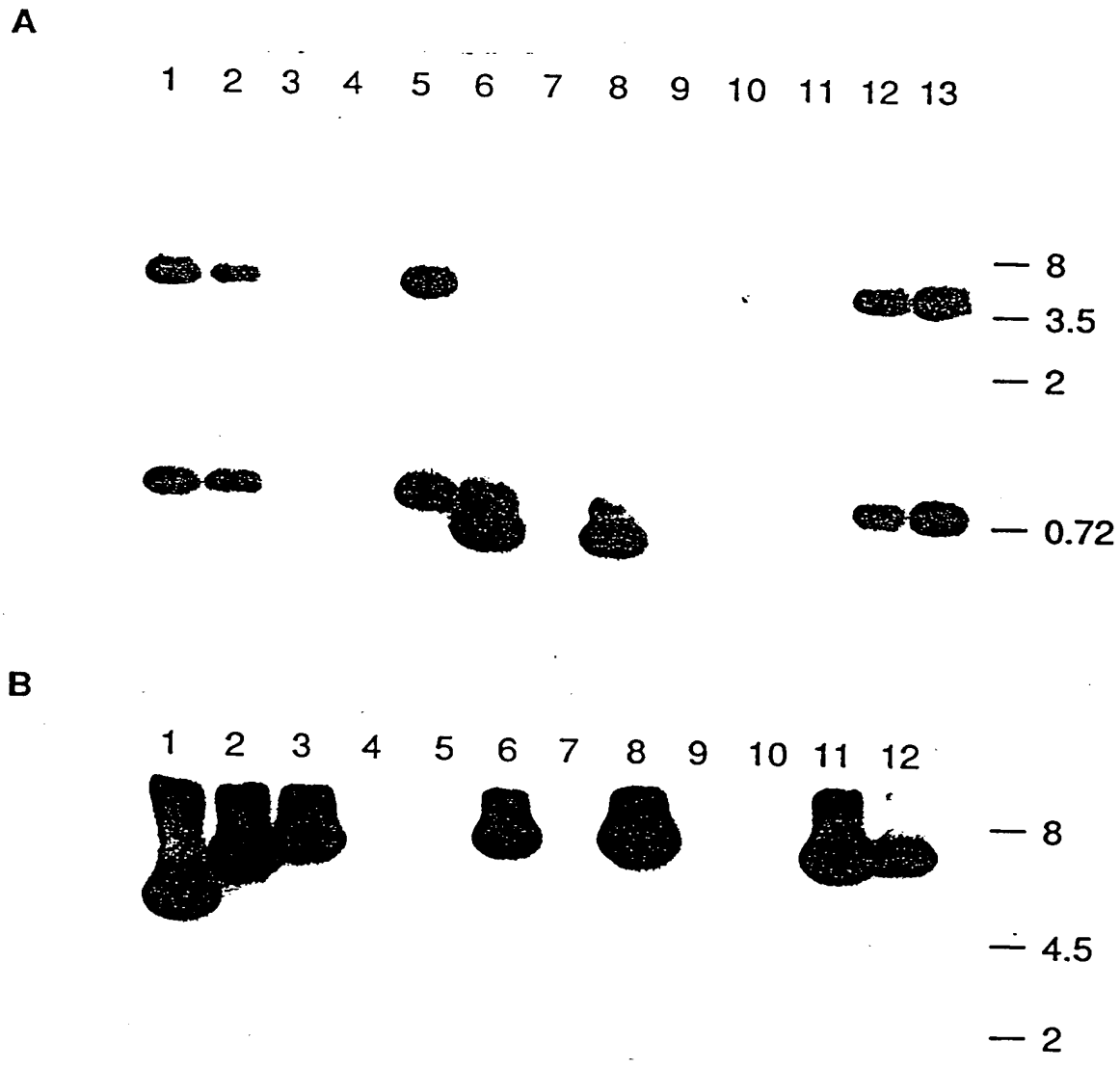


FIGURE 5

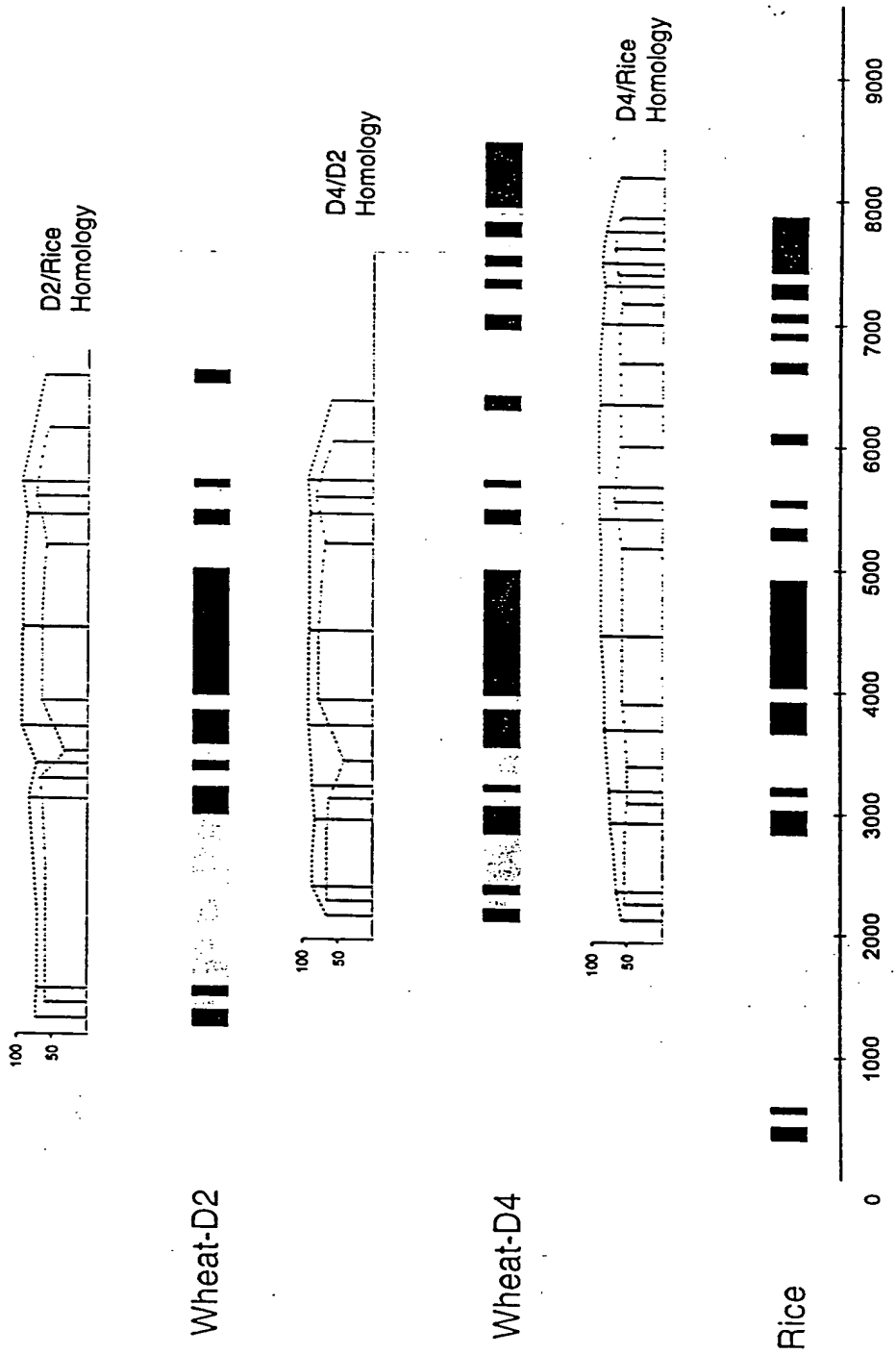

D2/Rice Homology
100
50
Wheat-D2
D4/D2 Homology
100
50
Wheat-D4
D4/Rice Homology
100
50
Rice
0
1000
2000
3000
4000
5000
6000
7000
8000
9000

FIGURE 6

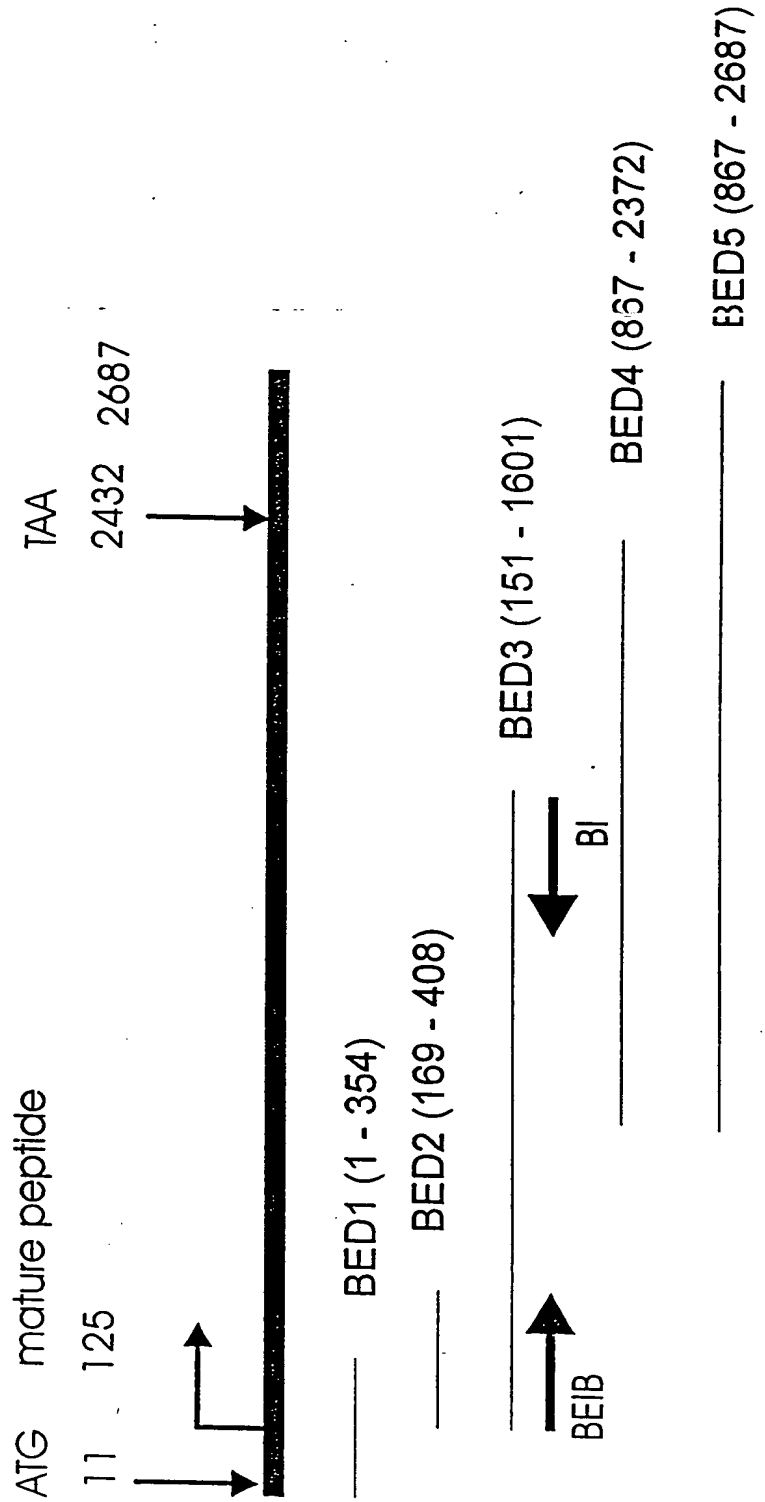

ATG
11
mature peptide
125
TAA
2432
2687
BED1 (1 - 354)
BED2 (169 - 408)
BEIB
BI
BED3 (151 - 1601)
BED4 (857 - 2372)
BED5 (867 - 2687)

FIGURE 7

```
  1  MLCLTAPSCS PSLPPRPSRP AADRPGPGIS AKSKFSVPVS APRDYTMATA
 51  EDGVGDLPIY DLDPKFAGFK EHFSYRMKKY LDQKHSIEKH EGGLEEFSKG
101  YLKFGINTEN DATVYREWAP AAMDAQLIGD FNNWNGSGHR MTKDNYGVWS
151  IRISHVNGKP AIPHNSKVKF RFHRGDGLWV DRVPAWIRYA TFDASKFGAP
201  YDGVHWDPPS GERYVFKHPR PRKPDAPRIY EAHVGMSGER PEVSTYREFA
251  DNVLPRIKAN NYNTVQLMAI MEHSILCFFW YHVTNFFAVS SRSGTPEDLK
301  YLVDKAHSLG LRVLMQVVHS HASSNMTDGL NGYDVGQNTQ ESYFHTGERG
351  YHKLWDSRLF NYANWEVLRY LLSNLRYWMD EFMFDGFRFD GVTSMLYNHH
401  GINMSFAGNY KEYFGLDTDV DAVVYMMLAN HLMHKILPEA TVVAEDVSGM
451  PVLCRSVDEG GVGFDYRLAM AIPDRWIDYL KNKDDLEWSM SAIAHTLTNR
501  RYTEKCIAYA ESHDQSIVGD KTMAFLLMDK EMYTGMSDLQ PASPTIDRGI
551  ALQKMIHFIT MALGGDGYLN FMGNEFGHPE WIDFPREGNN WSYDKCRRQW
601  SLSDIDHLRY KYMNAFDQAM NALDDKFSFL SSSKQIVSDM NEEKKIIVFE
651  RGDLVFVFNF HPSKTYDGYK VGCDLPGKYK VALDSDALMF GGHGRVAQYN
701  DHFTSPEGVP GVPETNFNNR PNSFKVLSPP RTCVAYYRVE EKAENLRMKE
751  LLLGAKLLLG TSMLKPLVSK TQQMVRRLLV PKRRLQGGDS SKKGINFVFG
801  SPDKDNK*
```

FIGURE 8

```
            1                                                        50
RSBEI'      .......... .********* *...:***pl lp******** **ag******
MSBEI'      .......... .****v*p** **tplp***r ***h***aa* pg********
D4cDNA      .......... .*****ap*c **sl..***p **pa****g* **s*......
PESBEII'    .......... .......... .......... .......... ..........
POSBE'      meinfkvlsk pirgsfp*f* pkv*sgas*n kic*psqh*t *lkf*sqers
D2cDNA'     .......... .****s**ll prp*a*.... ....****l* *******ggk
Consensus   ---------- MLCLTSSSS SP-S-APPK- SRS-ADRPSP GIIAGGGNVR
            51                                                      100
RSBEI       l..**v*... *p*****g** *tn***pa** rk****v*vv ***..*****
MSBEI       l..**l**qc ka***gv*** ****ataa*v q*d*****ak g**..*****
D4cDNA      .......... .......... ******p*s* prdy****a* *g*..gd***
PESBEII     .......... .......... ........mt d*ks**psv* **f..*nig*
POSBE       w..d*s*t*k *rv*kde*mk h*saisa*lt d**s***pl* ***kt*nigl
D2cDNA      rlsv*p***f ll**l****a ***sf*s*** rg**ia**.. tgygs*****
Consensus   ---SV-SVP- S-RRSWPRKV KSKFSV-VTA -DNKTMAT-E EDV--DHLPI
            101                                                     150
RSBEI       ********e* ****n**i** *****c**** ********** *********v
MSBEI       ********i* ********** *****gs**e n**s**s*** *********n
D4cDNA      *******ag* ****s****k ******s*** ********** **********
PESBEII     lnv*ss**p* ****k***** **h**k***e y****q**a* ******f*r*
POSBE       ln***t**p* l****h**** *v***m**** y**p****aq ******f*r*
D2cDNA      ****l**ae* ****d*trn* *i******** ***s****** **********
Consensus   YDLDPKLE-F KDHFRYRMKR YLDQKHLIEK HEGGLEEFSK GYLKFGINTE
            151                                                     200
RSBEI       *g******* ********** *******ak* *****k**** **k*******
MSBEI       *dg******* ******e*** ***d***a** *****k**** **k*d**k**
D4cDNA      nd******** ***m****** ********g* r*t**n**** **********
PESBEII     *dgis***** *******i** ***g*****l h****q**** **q*pdad*n
POSBE       *gci****** *****dev** ***g****** m****q**** ****pd*ds*
D2cDNA      hg*s****** ***e****** ********g* **a**n**** **********
Consensus   --ATVYREWA PAAQEAQLIG DFNNWNGSNH KMEKD-FGVW SIRISHVNGK
            201                                                     250
RSBEI       ********** ***r**g*a* ********** **f******* **********
MSBEI       ********** ***l*.g*** ******l*** ********** **********
D4cDNA      ********** ***hr*d*l* ********** **f******* **********
PESBEII     ********r** ***k*sd*** ********k* ****ptr*a* *****y****
POSBE       *v******r** ***k**n*** ********k* **a**t**a* *****y****
D2cDNA      ********** ***r*.h*** **q******* ***t**es** ****l*****
Consensus   PAIPHNSKVK FRF-HG-GVW VDRIPAWIRY ATVDASKFGA PYDGVHWDPP
            251                                                     300
RSBEI       ac******** ********** ********** ********** **********
MSBEI       a****t**** **s**a**** ********** k*a******* **********
D4cDNA      sg******** **r******* ********** r********* ********k*
PESBEII     l****q**** *****k**** ********ss **r*ns**** **d******e
POSBE       p****h**y* *****r**** ********ss **r*ns**** **d*****k*
D2cDNA      s******n** ******v*** ******v**g kl*ag***** p*****cl**
Consensus   -SERYVFKHP RPPKPDAPRI YEAHVGMSGE EPEVSTYREF ADNVLPRIRA
```

FIGURE 9

```
            301                                                       350
RSBEI       ********** ********** ******.*** ********** **********
MSBEI       ********** ********** ******.*** ********** **********
D4cDNA      ********** *****ilcf* w******.*** ********** **********
PESBEII     ********** ********** w****kp*** ******g*** **********
POSBE       ********** *******g** ******.*** ****y*n*** **********
D2cDNA      t********g *****ds*** ******.*** ********** **********
Consensus   NNYNTVQLMA- IMEHSYYASF- GYHVTN-FFA VSSRSGTPED LKYL-DKAHS
            351                                                       400
RSBEI       ********** ********** *********u *h******t** **********
MSBEI       ********** ********** ********** *******a** **********
D4cDNA      ********** *****s*m** *********n *******t** **********
PESBEII     ***n****** ********** ********** s*q****a** **********
POSBE       ***q**v*** ********** **********q u*******u*** **********
D2cDNA      ********** ********i* ********** ul*****yt** k***u***ug*
Consensus   LGLRVLMDVV HSHASNNVTD GLNGYDVGQS TQESYFH-GD RGYHKLWDSR
            401                                                       450
RSBEI       ********** ********** ********** ********** *****k*****
MSBEI       ********** ********** ********** ********** *****v*****
D4cDNA      ********** ********** ********** *********n ******s*a*
PESBEII     *******ks. s********** ****k***** ********** ******a****
POSBE       ********** ********** ****n***** *********v **********
D2cDNA      ********** ********** ********** *v********n *n****s*n*
Consensus   LFNYANWEVL RFLLSNLRYW -DEFMFDGFR FDGVTSMLYH HHGINMGFTG
            451                                                       500
RSBEI       ********** ********** *******l** ********** **********
MSBEI       **q******* a********** *******l** ********** **********
D4cDNA      ******g*** ********** *******i** ********** *******s**
PESBEII     d*n****e** ********** **s*v*di** ***d****** ***g*g***s
POSBE       **n****ea* ********** **n*i**i** ********** ***g*g***s
D2cDNA      *****ig*** n***f***** *******l** **i****v**** **********
Consensus   NYKEYFSLDT DVDAVVYMML ANHLMHK-LP EATVVAEDVS GMPVLCRPVD
            501                                                       550
RSBEI       ********** ********** *******rk* ****.*vq** **********
MSBEI       ********** ********** ********** **g*.*ah** **********
D4cDNA      ********** ********** *******l** ***a.*ah** **********
PESBEII     *v********* ******k*** *****k**** **k*.*sln* **********
POSBE       ********** ******k*** *****n*e** **k*.*tss* **********
D2cDNA      ***l*****q **t******* **e**g*qq* ***sv*sq** *****p**f*
Consensus   EGGVGFDYRL AMAIPDRWID YLKNKDDSEW SMSE-I--TL TNRRYTEKCI
            551                                                       600
RSBEI       ********** ********** ******t*** *********n **********
MSBEI       ********** ********** ******t*** ********** **********
D4cDNA      ********** *****m**** ******t*** ********** **********
PESBEII     s********** ********** **e***ss** c*tml***** ***s*h****
POSBE       ********** ********** ******s*** c*td***v** *****h****
D2cDNA      ****rqnh** **s**m**** **w*t*s*** a*d*d***** *a*********
Consensus   AYAESHDQSI VGDKTIAFLL MDKEMY-GMS DLQPASPTID RGIALQKMIH
```

FIGURE 9 (cont.)

```
            601                                                        650
RSBEI       ********** ********** ********** ********** .*********
MSBEI       ********** ********** ********** ********** .*********
D4cDNA      ********** ********** ********** ********** .*****s*i*
PESBEII     ********** ********** ********** **g******* lt**n****n
POSBE       *f******** ********** ********** ********** .***n*a*s*
D2cDNA      *********s **k*****.. .......... .......... ..........
Consensus   FITMALGGDG YLNFMGNEFG HPEWIDFPRE GNNWSYDKCR -RQWSLVDTD
            651                                                        700
RSBEI       ********** *********e ********** ******k*** **********
MSBEI       ********** *********r ********** ********** **********
D4cDNA      ********** ********** ********** *******k** **********
PESBEII     ********** *r***l**** **i*a*t*** **st*n**** **********
POSBE       ********** *r***s**** ****a*g*** **s*d**n** **********
D2cDNA      .....***** v**vdtps** c******n*t a*h******g sa*tk*....
Consensus   HLRYKYMNAF DQAMNALD-K FSFLSSSKOI VSDMNEE-KV IVFERGDLVF
            701                                                        750
RSBEI       ******n*** k********* ********** **v******* **********
MSBEI       ******k*** ********** ********** **v******* **********
D4cDNA      ******s*** ********** ***k****** **m******* aqyn******
PESBEII     ******en** ********** ********** *te******* ***a*q****
POSBE       ******kn** ********** ********** *wc******t **********
D2cDNA      .*thlrsgc* *p.....s** stssc**.... .*gpsnqspf skpfig*pgc
Consensus   VFNFHP-KTY EGYKVGCDLP GKYRVALDSD AL-FGGHGRV GHDVDHFTSP
            751                                                        800
RSBEI       **m******* *****..... .......... .....***** **********
MSBEI       ********** *****..... .......... .....***** **********
D4cDNA      ********** *****..... .......... .....***** **********
PESBEII     ********** *****..... .......... .....***** ****h***v*
POSBE       ********** **g*qipskc cllrehvwli telmnacq*l kitrq*f*vs
D2cDNA      ifcc*lfkge *.......... .......... .......... ..........
Consensus   EG-PGVPETN FNNRP----- ---------- -----NSFKV LSPPRTCVAY
            801                                                        850
RSBEI       *...****dr ****rggava s**i.vtey. ....***e*t sgetisggwk
MSBEI       *...****ag agr**hakae t***sp*es. ....***k*s ra....*ske
D4cDNA      *...****ka *n***ke**l ga*l*lgtsm lkp**sk*qq mvrrllvpkr
PESBEII     *...*****q **snnpn*gs *ee**a*adt dvar*pdvs* e*..ed*nld
POSBE       *yqqp*sr*v trnlkiry*q *sv**tnacq klk*trq*f* v*yyqqpilr
D2cDNA      .......... .......... .......... .......... ..........
Consensus   Y---RVDER- EELR---LL- -GKTL-A--- ----IDVTA- -S----S---
            851                    876
RSBEI       gs*kd*cg** *mk***r*** e*c*d.
MSBEI       dk*atagg** *wk*arqp** q*t**.
D4cDNA      *lqgg*ss** *in***g*p* k*n**.
PESBEII     ***dnsedav dagilkver* vvgdn*
POSBE       **trklkdsl stnist*... ......
D2cDNA      .......... .......... ......
Consensus   R-E--D--KK G--FVF-SSD -D-K--
```

FIGURE 9 (cont.)

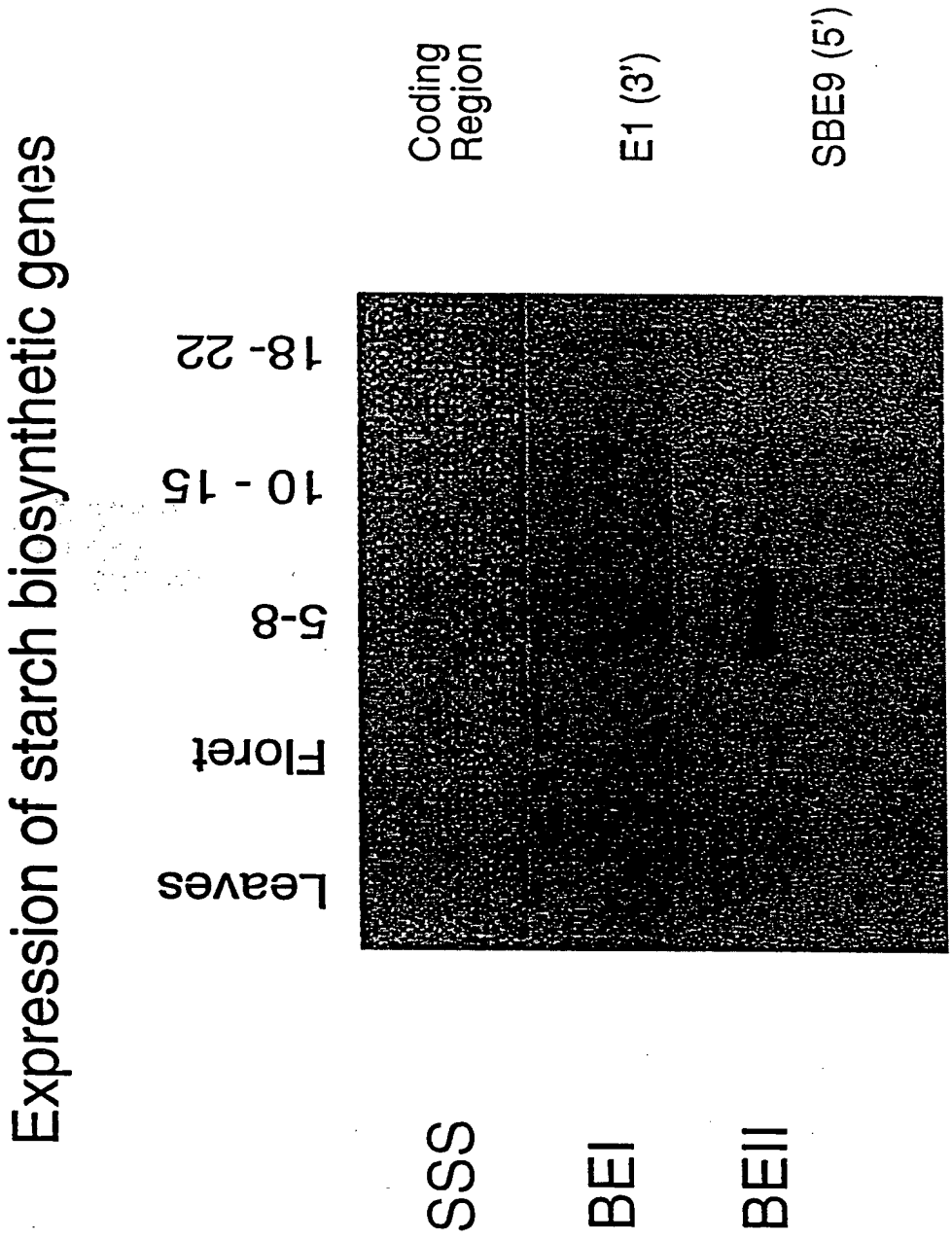

Expression of starch biosynthetic genes
Leaves
Floret
5-8
10 - 15
18- 22
Coding Region
E1 (3')
SBE9 (5')
SSS
BEI
BEII

FIGURE 10

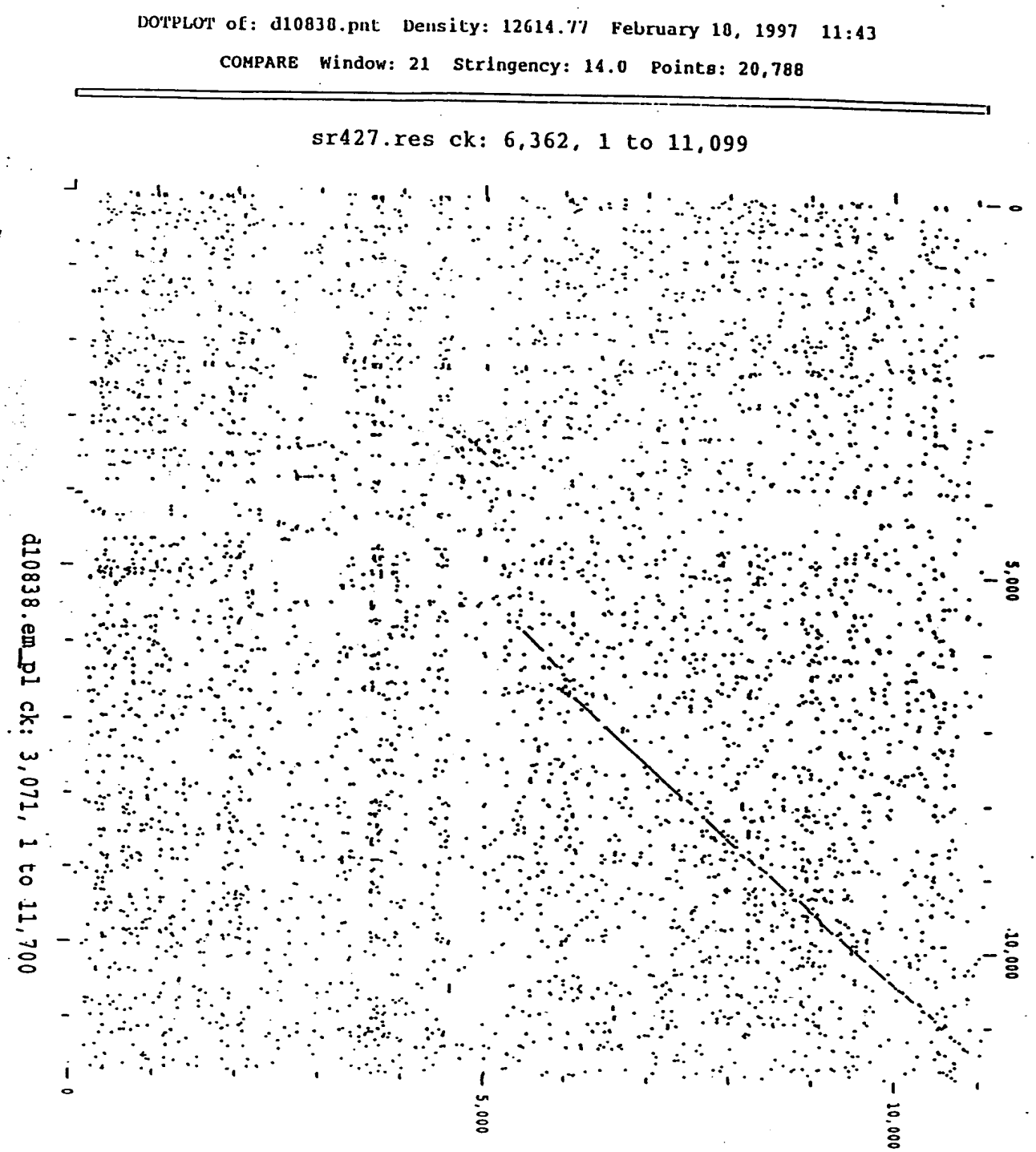

DOTPLOT of: d10838.pnt Density: 12614.77 February 18, 1997 11:43
COMPARE Window: 21 Stringency: 14.0 Points: 20,788
sr427.res ck: 6,362, 1 to 11,099
d10838.em_p1 ck: 3,071, 1 to 11,700
0
5,000
10,000
0
5,000
10,000

FIGURE 11

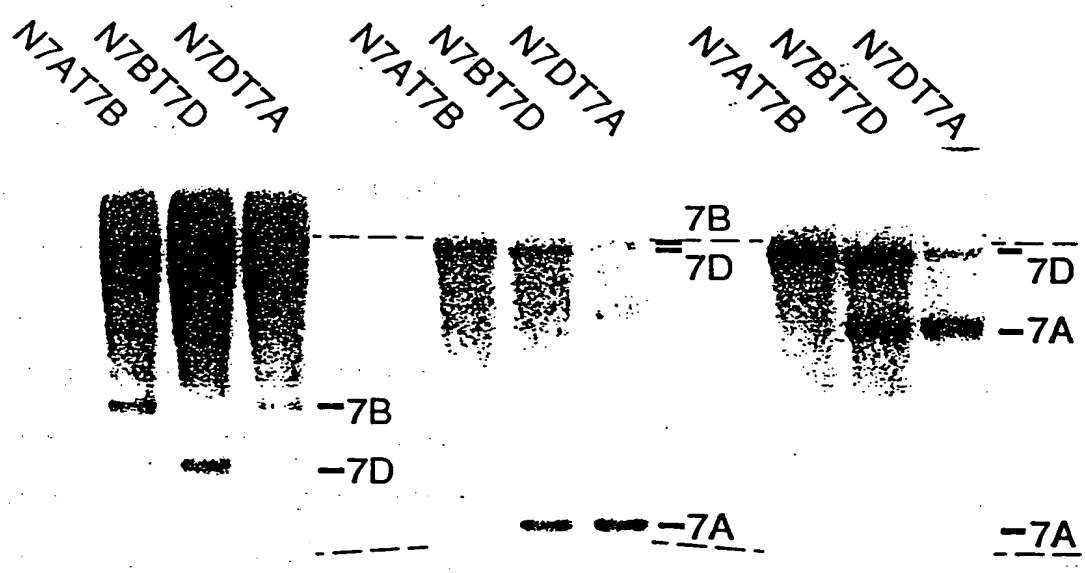

N7AT7B
N7BT7D
N7DT7A
N7AT7B
N7BT7D
N7DT7A
N7AT7B
N7BT7D
N7DT7A
7B
7D
7D
7A
7B
7D
7A
7A

FIGURE 12

```
   1  GGGTGGCGGG TCGGGCGGCA AGGCGCGGGG CGGCGGGGCG GCNCGGGGCG
  51  GCNGCGGCGG CGCGGGCGGC AGCGGCGGCT AGGGTTTCGC GGCGGCGGCG
 101  ACTTGGGCTG AGGCGGGGCA CGGGCTGCGG CTTTAAAGGC CGGCCAGGCT
 151  GAGGTGTCCG GGTCGGACAC GGCCCGTAAG GCGGTTGACT TTAAAAAATA
 201  ATAATTCGGA CATGCAAAAA AGTAAGAAAA GAAATAATAA ACGGACTCCA
 251  AAAATCCCGA AGTAAATTTT TCCCCATTCT TAAAAATAAG CCGGACAAGA
 301  TGAACATTTA TTTGGGCCTA AAATGCAATT TTGAAAAATG CGTATTTTTC
 351  CTAATTCGGA ATAAAATCAA ATAAAATCCA AATAAAATCA AATATTTGTT
 401  TTTAATATTT TTCCTCCAAT ATTTCATTAT TTGTGAAGAA GTCATTTTAT
 451  CCCATCTCAT ATATTTTGAT ATGAAATATT TTCGGAGAGA AAAATAATTA
 501  AAACAAATGA TCCTATTTTC AAAATTTGAG AAAACCCAAA TATGAAAATA
 551  ACGAAATCCC CAACTCTCTC CGTGGGTCCT TGAGTTGCGT GAAATTTCTA
 601  GGATCACAAA TCAAAATGCA ATAAAATATG ATATGCATGA TGATCTAATG
 651  TATAACATTC CAATTGAAAA TTTGGGATGT TACATATAAC TCAAATTCTA
 701  TAATTATGAA CACAGAAATA TTAATGTAGA ACTCTATTTT GTTTTGAAAT
 751  TGTATTATTT TTTAGAATTA GTCTAGAGCA TTTCGTGAAC TTGAATCAAA
 801  CCTTTAAATA AAACAAAGCA TAAAAATGAC AAATTCACAT ATGAAATAAC
 851  TTGTGTTACA TAGATTTATT ACAATAGCGT TGTATGTGTG TATGTGTGCG
 901  TGAGTGCCTA TGGTAATATC AATAAATATC TTGATAGATG TTTCTACAAT
 951  TCACGGGTCT AACTAGTAAT GCAATGCAAT GCATGCTAAA AGAATAGAAC
1001  CTTAGTTTCA TTTAACTAAC AATTTTCAAA TGTATGAGTT GCCAACAAGT
1051  GGCATACTTG GCACTGTTTG TTTGTTCATT TTATGGAAAG TTCTTCTCTT
```

FIGURE 13a

1101 TTTACATGGT TTAGATTCCA GCATGTAGCC ACAAAATATG ATTGTCAAAA

1151 GATAATACCT CATAATACAA TTCCACTAAA GTCACCTAGC CCAAGTGACC

1201 GACCTGATCC TGAAATAAAA TCAGAAGATT TGGTGTCATC ATCATGACAA

1251 CAAATTATTA GGCGGTAGAT CTTGTGGTAG TACTCATGAT GTAAAATTAT

1301 CAAGAGGGAG AGAATGTATG GAGATTTATG TGAAGTACAT CGTACACCAG

1351 ACATAGTTGA CACATCGATT TTTTAAGATA CATTTGGACG CGCCTTGTGG

1401 GAGTGTAAAG TACTACCATG TATTAGAAGA GGTGAAATGA GAAATGCCAT

1451 AGCTAGCAAG TAGGCCTAGT TAAGGAAATT CTTCCTTAGA NTCCCCTTCT

1501 CCCGAAGAGT GAAGTGCTTC AACTAAAGGT TAGACCCACT TAAAAAATGT

1551 CACTTTGAAT CTTTGCTTCC CTTGTCGTAA TCCTGTGCAT TTGTAGGTCC

1601 CTCGGATCTG AGCCCTTTCT CCAAGCCCTT CATTGGATTC CCCTGGATGT

1651 CTTTTTGTTA CATTTTATTG AAGTGAGAGT GAATTATTAT ATGCCCATAG

1701 GAGGTGGGAT ATAAAGGCTG TTGGTATTCT GCACCATACA TGCTAGAGTA

1751 GGGAGGAGAG GCTGGTGCAT GATACATGGT GGACTAGCCC ATATATTTAC

1801 CCCTCCCCCA CCCACNTAAC AAGTTTTTTT NTATTAGGTC TTCATCCTCT

1851 GATTTGTTTT TCTGTTAGCC CATTCTTCAT CATGGACTTA TTAATCATGA

1901 TTAGTTTCTT GGATTTTTGT TTACTTGACT TGAATTTGAC AATGTGCCTC

1951 ATATATGGCA TGTGGGACTG ATAGGAAGAT ATATTCTCAC AACATTAACT

2001 TAAAAAGGAT TATTTTTTTG GTGCAGTCGT AAAGAAAACT ACTTTCTTTT

2051 ATGCTAAAAG TTATTCAAAC ATAGATTTAT AAACAAAGGA TATCACCATG

2101 CATGACCATG CGCTCTCTCA TGTTTACTCT AGAAACCATA TATCTCTTTG

2151 TTGCAAAATA TTTAATCTAT CCTCCTTGTT TCTGGGAATG AGTCGGGGAA

FIGURE 13a (cont.)

2201 GGTAATCTTA GGGAAGGTTA AAGTGAGGCA AGTAAGAGCA ACTCTAGCAG

2251 AGTCGCGATA TGCCCAATCG CCATAATGCC AATATGGCAT TTTTGGCCCA

2301 AAATGGCACT TCAGAAGAGT CACCATATCC CTTCGGATAG CCATAATTTA

2351 GGGAGCTCGC TCCACAAACA AGCTTCGAGC CTCCAAATAT GGAGGCCATG

2401 GATTCGTTGT TTGGCACTCA CTCCATATCC AACCGCAAGC GCATGCATGA

2451 GGGAAGTTTT AGCTTCTTCC TCCTTGCGCC AACGCCGGGA TTTTACACAG

2501 CGCATTACAG GTACATGAAC CAGCATGCAC AGATAATCAC CGACGAGTGG

2551 GGTGACAAGA AGGATAAGCA CCCTCCCATT AGTGGTGCGC CCACTCCCCT

2601 CAAATTCATG AGGCAGCCAT TTGGATGGTC ATCGCGTGGC ATAAGCTCCG

2651 ACTATAAAAT CTCAACGGCA TCACCAAAAC CATAGCTGCC GCCTCCCCCT

2701 TCCTCGGCAT CACCTCCCCA AGACATCTCC TCCCCTCTAT GCCACAATGT

2751 CATCATTATG GAGAGACACA ACNTACTGGN TAAACCGCAT ACCCAATCAT

2801 GGTTTACCGG CAGTGCGAAC CCCACCTTCC TCCCACGATG GTAGGATATT

2851 CTCCTCCTAG AATGGCGCGT GTGGCGCTTC CTCCTCCCGA GGCTGATATG

2901 TCGGCTCCCA TGATGGCGTG CATCATTGAT TTGGCGCTTC GGGTCCATCA

2951 TACATGTTAA CGAGGTCATC CCCATTGATG TCGTTGGTCC CCTTGCCCCC

3001 CAGTCGGATC CTGAGGACCC GTTCGATGTC GCAATGCGAC TCTCCAAACT

3051 CAAAGCTCAC AATGAGGAGT ACGTCCTCTA GGAGTTCCGC CCCGCAACCA

3101 TCTATAAGGA GGAGCAACGA TAGCTCTCCC CTACGCCTTC CTCGACGATC

3151 TCTCTTAGGA GGACAACGGC TAGACGACGG CGGCGGCGGC GAAGGTACTG

3201 CAGGTAGTAG AACATAGCAA TGTCGAATGG CGACATTGCA TATTTTGAAA

3251 ATGTCGCTCA ACGACTTTTG AAGTCGCAAA TAAAATGTAG TGTGACTACT

FIGURE 13a (cont.)

```
3301  TTTGGCCAGC AATATAAGTT TATCACATTT GATAATGATT TGAACCGGTG
3351  TGGTTCAACT AAATGTACCA TAAATTGAAC ATACAAATTT TTAGCAAATG
3401  AAAAAAGAAA CAAGTAAGAC CACAAATATG AAAGCCGCAT ATCGCGACTA
3451  TGTGTTTGAG CCGCAGCTGC CAAGTACATA TGAAGCGTAC TCCATATGAC
3501  ATACGACAAC CATACATATG AAGACTCTAC TAGAGTTCTC TAAGGCCGCT
3551  TTTAGCGCCT TTCGTGCAGT GGTGCCCATA GGGAGTGAGG GTAGTTGGAC
3601  TGTTCGTTTC CCCTTTTTTC ATTTCTTTGA AATCTATTTT ATTTTTTTTC
3651  TCTTTTGTAG GTTTCCCAAA TTTATATACC ATTTTTCTGT TTCTCGCTAT
3701  TTTTTGTTGT TATATTCTAG TTTCATATTT TTCTATTATT AATTTGTGTC
3751  TCTTATGAGA AGTCCAGACT TGCATATGGA GGTGCACACA CAAACATATA
3801  AAGTATAAAT ACTAACTTGA GAAGTATGTT TGCGTGGTCA AAAAAACATC
3851  ATCAAAACCT GCCAATATGA GATATAGTTT TGAATATATC AATATGAGCA
3901  ACGCAACCAT TTAAAATGTG AACAATTGTT TTTTTAGAAA AAATATAAGA
3951  AATAACTCCA ACCCAGCCAA ACCACATGCT ATACACTTGC TCCATATGAA
4001  ACCATGTTTG CTATTGGGCA GTTGCCTGAA ACCGAAAGTA ATGTTAGCCG
4051  TTTTTCTATT CAAAGAAGAA GGAGAGTCGA GGTGACGCGA TGCTTAGACG
4101  NTGAGATGGG GATGACCACA ACGTCCCTAC AGAGACCTCA CCGGAGATGG
4151  GGACATTGCA GTTGACACGA GAGCGGTGAG GGGCTGCGAT GCGTGTGCGG
4201  CAACATGTGG CGAGGCGGAC GTCGGGCTGG CAGGTAGGGG GGAGGGGGAA
4251  GGACCGGGGG AGGAAGAAGA GGAGTAGCCT GCAAAACATG GTACACCAGT
4301  TTTCTGCCCT ACGAAAACCT CATTTCATTC CCCCACCCTG ACAAGCAACA
4351  ACCAACCATC GCAGTCCCAC ATGTCCCTCT GGTCTTTGCA AAAAGTAATT
```

FIGURE 13a (cont.)

```
4401  GTTCTTGCTG GACAGCGCAA AGAGTAAACT TTTGTTAGTT TTCATTTCTA
4451  GAAAAAGCAA TCCTTTTATA GTTCTTTTGT GAAAGTAATG CTTTTATAGT
4501  GATTGGGATG TTCTTTTAGA GCAAATATCT TCTTTTTTTT TTAGGGAAAA
4551  GAGCAAATAT CTTCCACTTT TCACAAAACT GACGAAGGCT GAAAGTGGCG
4601  AGACANGTGA GGGCCCATAG CTTTCGTCCG GCCCAGCGGC GCACGACCGT
4651  CCACGTGCAC CCCGGCCCTC CCGGGCCCGC AGATCCGNTT CTCCCTCGCC
4701  CCCGTTTCCC CCTCCCTCCC TCTCGTTGCT TCCACTCCAC TGTTCTCCTC
4751  TTCCTGTCCA AAGCGGCCAC GGACCGGAAA AAAATCACGC CTTTCCGTTG
4801  GGTCTCCGGC GCCACACTCC TCCTCCGGCC GATATAAAGC GCGCGGGGCC
4851  ACGGGCCCGG CGCAAAATGG GATTCCCGTC CGCCGCCATG GAGGAAGATG
```

FIGURE 13a (cont.)

```
   1  ACGGGCCCGG CGCAAAATGG GATTCCCGTC CGCCGCCATG GAGGAAGATG
  51  TTCTGCTTCA CCGCCCCTTC CTGNTCGCCA TNTCTCCCGC CGCGCCCNTC
 101  CCGTCCCGNT GCTGACCGGC CCGGACCGGG GATCTCGGTG AGTCAGTCGG
 151  GATCTTCATT TCTTTTCTTT TCTTTCGTTT CCGGCNTCCG TTCTGCCGGG
 201  GTTTCCCTGA TGCGATGCCG CGCGCGCGCA GGGCGGCGGC AATGTGCGGC
 251  TGAGCGCGGT GCCCGCGCCC TCTTCGCTCC GCTGGTGTGG CCGCGGAAGG
 301  TGAGCCCTCT CCCCTGTCTA CCCAGATTTG CGACCGTGAT CCCCTGTTGT
 351  CGCCGGGCAA ACGGAATCTG ATCCACGGTG GTTATTGGAA ATAGTATATA
 401  CTACTAATAA ACTTGAGGCT GGGATTCGTC CACTGAGGAA CAAGTGGATG
 451  CGATTTCGAT TGGATTTCTC TGCTTTATGC GATCCGTACG CAGAATATCC
 501  CTCCTGCAGT GTCTCAACCG TATTACTGGA TGTACAACCC AAATGTGTAT
 551  AATCTGTGCT GAATGTATCA ACCAATAATT GCTGCATTGT GAAAACATAA
 601  TCCTGTGTTG TGTCTCTACT ACTTGTTCAG TCCTGATCTG CCGCTTATCC
 651  TAACTTTTGT TCATTTATGG AAGGCCAAGA GCAAGTTCTC TGTTCCCGTG
 701  TCTGCGCCAA GAGACTACAC CATGGCAACA GCTGAAGATG GTGTTGGCGA
 751  CCTTCCGATA TACGATCTGG ATCCGAAGTT TGCCGGCTTC AAGGAACACT
 801  TCAGTTATAG GATGAAAAAG TACCTTGACC AGAAACATTC GATTGAGAAG
 851  CACGAGGGAG GCCTTGAAGA GTTCTCTAAA GGTTAGCTTT TGTTTCATGT
 901  GTTTGAAACA ATAGTTACAT CTTGTGGCGT CCGCAGCACA AAAGACATAA
 951  TGCGACTCTG TTTTGTAGGC TATTTGAAGT TTGGGATCAA CACAGAAAAT
1001  GACGCAACTG TGTACCGGGA ATGGGCCCCT GCAGCAATGT AAGTTCTAGT
1051  GTTGTCACGC AACTAATTGC AATGGTCGTT GGTTAACTTA TGAAGTGCTG
```

FIGURE 13b

```
1101  ATGAAACTGT CTTAAGAGTT TATGGCTTGT CTTTTCTGAT TCTAGCTAGT
1151  AAAGAGTAGA TAAATATGAA ATATGTTTTC CCTTTTCTAG TTATGGTCAT
1201  GGTTGGCTGG TATTCATTTC TTTTATGGCA ATACTTGCTT CTAACTATCT
1251  TTAGTAGATT CATGTATTTA CTTGTGAGTC ATTACTTTAT GGGTGTAGGG
1301  ATGCACAACT TATTGGTGAC TTCAACAACT GGAATGGCTC TGGGCACAGG
1351  ATGACAAAGG ATAATTATGG TGTTTGGTCA ATCAGGATTT CCCATGTCAA
1401  TGGGAAACCT GCCATCCCCC ATAATTCCAA GGTTAAATTT CGATTTCACC
1451  GTGGAGATGG ACTATGGGTC GATCGGGTTC CTGCATGGAT TCGTTATGCA
1551  TCCACCTTCT GGTGAAAGGT CTACTTTTAG TGGCTCGAGA GCAAGAAATC
1601  TAAGTAAAAC CCACACAATT AACTTACATT AATGTGGAGA CATGATACTT
1651  TTATTGCTCG TTTTGCAGGT ATGTGTTTAA GCATCCTCGG CCTCGAAAGC
1701  CTGACGCTCC ACGTATTTAC GAGGCTCATG TGGGGATGAG TGGTGAAAAG
1751  CCTGAAGTAA GCACATACAG AGAATTTGCA GACAATGTGT TACCGCGCAT
1801  AAAGGCAAAC AACTACAACA CAGTTCAGCT GATGGCAATC ATGGAACATT
1851  CCATATTATG CTTCTTTTGG GTACATGTTG ACGAATTTCT TCGCAGTTAG
1901  CAGCAGATCA GGAACCCAGA AGACCTCCAA TATCTTGTTG ACAAGGCACA
1951  TAGTTTAGGT TGCGTGTTCT GATGGATGTT GTCCATAGCC ATGCGAGCAG
2001  TAATAAGACA GATGGTCTTA ATGGCTATGA TGTTGGGCAA AACACACAGG
2051  AGTCCTATTT CCACACAGGA GAAAGGGGCT ATCATAAACT GTGGGATAGC
2101  CGCCTGTTCA ACTATGCCAA TTGGGANGTC TTACGATTTC TTCTTTCTAA
2151  TCTGAGATAT TGGATGGACG AATTCATGTT TGATGGCTTC CGATTTGATG
```

FIGURE 13b (cont.)

2201 GGGTAACATC CATGCTATAT AATCACCATG GTATCAATAT GTCATTCGCT

2251 GGAAGTTACA AGGAATATTT TGGTTTGGAT ACTGATGTAG ATGCAGTTGT

2301 TTACCTGATG CTTGCGAACC ATTTAATGCA CAAACTCTTG CCAGAAGCAA

2351 CTGTTGTTGC AGAAGATGTT TCAGGCATGC CAGTGCTTTG TCGGTCAGTT

2401 GATGAAGGTG GAGTAGGGTT TGACTATCGC CTGGCTATGG CTATTCCTGA

2451 TAGATGGATC GACTACTTGA AGAACAAAGA TGACCTTGAA TGGTCAATGA

2501 GTGGAATAGC ACATACTCTG ACCAACAGGA GATATACGGA AAAGTGCATT

2551 GCATATGCTG AGAGCCATGA TCAGGTATGT TTTCCCTCCT TTGTCGCTGT

2601 GCGTGAGTAT GTGTTCTTTT TTTATGGGGC ACTGGTCTAA GAACATACAG

2651 TTCAAAGGTG AGACACTTTC TTTGCCTGGT AGACAAATTT GAGAAATAAA

2701 CATTTCGCTT GATGACTTTT AGTTGCTTCA CAAGTTCGAA TTAAGTTAGT

2751 TATATTCTGA TAACTAGTGA TAGTACCCAC TAACCAGCTA TTACGGACCA

2801 TGTAAGAATG TCCGAAGACT GCAGTTATAT ATCGTTGACT TTGTGTTCAT

2851 CTATTGAAAC AACTTAGTAG TTAACTTTCA CGCAAATTTT CAGTCTATTG

2901 TTGGCGACAA GACTATGGCA TTTCTCTTGA TGGACAAGGA AATGTATACT

2951 GGCATGTCAG ACTTGCAGCC TGCTTCGCCT ACAATTGATC GTGGAATTGC

3001 ACTTCAAAAG GTTCGATTCG TTTTAAGTAT TCCTGAATTT GATGTTCTAG

3051 TTCCAGACGA GTATTGTAAT GTTCGTTGTT ACTCAGAGTT CTGCTTAGTC

3101 CTTGAAGATA ATGTATTCCA GTCCCTTTTG GTACATTTGG CTTATTTTGT

3151 TACAAATATT TCAGATGATT CACTTCATCA CCATGGCCCT TGGAGGTGAT

3201 GGCTACTTGA ATTTTATGGG TAATGAGGTA ATATCTGGTT ATCTGTCAAA

3251 ACTTATTTCT GATCAATATG TTTCGGGATT CCCTCGAAAA AAATCCTTTG

3301 GGCAGGGCGA AAAGTTTAAA CATCTGTTTT CTATGATAGC CAAGTACTCC

3351 CCAGCTATTT CCATGTTATC ACGTATCATT TAGCTGTGCC GGTAGTTAAT

FIGURE 13b (cont.)

```
3401  CTTTATTCTA ATTCATTGTT GTTTTTTAGC GTGGCAGTCT ATTGTTGGAT
3451  CCTCTTATTC CAATTACATA TATGCCGACA TCACACACTT ATGAATATTC
3501  CCTGTTTAAA AGATTTTTAT TTTATACCAA TGTTTCTCCG TAAATGATGC
3551  AAACATGATA GAGATGTTAG CATGTCTTTC TTAACCTACT CATGTTTTAC
3601  ATATCACGAC AAGCTTCTTG CAGAAAATCA GCAGTATATG GCAAATTGCT
3651  GCAACCTGAC AACGTTTATA TCTGTTTTCT AACTCATACT GACGGTGCAA
3701  TTTCCTTTTA GTTTGGCCAC CCAGAATGGA TTGACTTTCC AAGAAGAAGG
3751  CAACAACTGG AGTTATGATA AATGCAGACG CCAGTGGAGC CTCGCAGACA
3801  TTGATCACCT ACGATACAAG GTTATGCCTA TGTATATTTT TACAGTTTCT
3851  GGTCTGGTAG CTCTCTTGGG ATCTTGACCT CACTTAGTTC CTTCATCTCT
3901  GACTGTAGCT TATTTACACT GTGTTCCAAC TTCTGTCTTG TGGATAAATT
3951  CTCCCTTCTA ACGTTTCATA TTAAGCCTTT CAAACTAAAC TAAATTGCTG
4001  ATCTACTACT AGTTGCTCAG TACGATGACC AAATCTTGCC TGTGGTAACC
4051  TAGTAATTTT CTTGATTCTT ACACATTAGT GATATGCAGT GCATACATTA
4101  TCCATATAAA TTGACATTGC AATTTCCCAA ATATTATTTG AAGGCTGTGT
4151  TCTTTTGTTA ACAGGAAGTT ATTTTCTCTG CATCTGATAA ATAATAATAG
4201  CCTTTCACGA TTTTTCTCAT ATTTTATCCA ACTTTTCTGC ATTCAAGCAT
4251  TTTTTGTTTC TCGCCTAACA TATATAATTT GAACAGTACA TGAACGCATT
4301  TGATCAAGCA ATGAATGCGC TCGACGACAA ATTTTCCTTC CTATCATCAT
4351  CAAAGCAGAT TGTCAGCGAC ATGAATGAGG AAAAGAAGTA GTTAACTATA
4401  CAATGTTTAG TCAGGGCAGC TGTTGCATCA TTTGATTCAC TCCTACTCTT
4451  AAGAATAGCA ACTCTGACTT GTGCGTTTTA TGTTACCAAA TAAGTTGAAA
4501  CCGTATCTGT TTGATATGAA CCATTGTTGT CTCAAAATGG GCTATGGACT
4551  CAATCCAACT TCCTTTCCAG ATTATTGTAT TTGAACGTGG ANATCTGGTC
```

FIGURE 13b (cont.)

```
4601  TTCGTCTTCA ATTTTCATCC CAGTAAAACT TATGATGGGT AACTGATCTC
4651  TTGCAAGCTT TGCCTTTCAA TATTTCTTCT GCTTAATGAC TAATGTGCTT
4701  AATCTCGTTT CCACTTTTAA AACACGCAGT TACAAAGTCG GATGTGACTT
4751  GCCTGGGAAG TACAAGGTAG CTCTGGACTC TGATGCTCTG ATGTTTGGTG
4801  GACATGGAAG AGTAAGCAAT GTTAATGATG TTCAAGATCT GTTTTGCAAC
4851  ACTATGTTCT TCTATAGAAG GGGCCATCAA GGCTGCATCA GATAATCTTA
4901  TTTGCAGTGT TGATCTGTGC TGCATCGCAG GTGGCCCATG ACAACGATCA
4951  CTTTACGTCA CCTGAAGGAG TACCAGGAGT ACCTGAAACA AACTTCAACA
5001  ACCGCCCTAA CTCATTCAAA ATCCTGTCTC CATCCCGCAC TTGTGTGGTA
5051  ATGCTAATTA CTAGGAGGAT TTAGTAACAA TAAATAAATA ACAGCAAAAG
5101  ATATCTGCAG TACGATCTCA CAAAATGCTC TCTTGCCAGG CTTACTATCG
5151  CGTCGAGGAG AAAGCGGAAA AGCCCAAGGA TGAAGGAGCT GCTTTCTTGG
5201  GGGAAACTGC TCTCGGGTAC ATCGATGTTG AAGCCACTGG CGTCAAAGAC
5251  GCAGCAGATG GTGAGGCGAC TTCTGGTTCC GAAAAGGCGT CTACAGGAGG
5301  TGACTCCAGC AAGAAGGGAA TTAACTTTGT CTTTCTGTCA CCCGACAAAG
5351  ACAACAAATA AGCACCATAT CAACGCTTGA TCAGGACCGT GTGCCGACGT
5401  CCTTGTAATA CTCCTGCTAT TGCTAGTAGT AGCAATACTG TCAAACTGTG
5451  CAGACTTGAA ATTCTGGCTT GGACTTTGCT GAGGTTACCT ACTATATAGA
5501  AAGATAAATA AGCGGTGATG GTGCGGGTCG AGTCCAGCTA TATGTGCCAA
5551  ATATGCGCCA TCCCGAGTCC TCTGTCATAA AGAAAGTTTC GGGCTTCCAT
5601  CCCAGAATAA AAACAGTTGT CTGTTTGCAA TTTCTTTTTG TCTTGCATAG
5651  TTACATGATA ATTGATGCAT ATTGCTATAA GCCTGGATTG CATCTTCTTT
5701  TGCTAATAAC TGCAGGGCCA AGAAAGCCTA GATTGTATCT TTTTTTGCTA
5751  ATAACTGCAG TGCTGGGGAA GCTTCAGTCC TTGTTTCCGT TCTCGAGACA
```

FIGURE 13b (cont.)

```
5801  AGGCGTCATG TTTGGCGCAC AAAGGTAAGC CATCATCTTA TCAAGTCCCA
5851  AAATTCTCTG GTTGAAAGAA ACCATCACTA ACTTGTTCCA GGTGTTGGTT
5901  CCTCCACAAC CAAAAGGCGA CCATCGTCGT CATCATCGCT CACAGCACTG
5951  ACCATCGAAG CCACGGTGGG CATGANAANT GCGCATCGCC CAAGACTTGG
6001  GACCGTTTCA AAANTATCAC AAACTGCCAT GGNCATCTTC TGCCAAAGGC
6051  TGCACTGCAC CTTTGGCATG AACAGAAGCA ANNCAGGGGC TTGGAACTGA
6101  ACNGCCGAAA ATAAAGTCAA NACCGGCTGG GCCGGATTGA AAGGGGAAAC
6151  GNCCAAAATC CACTTNAATT TGAATGGAAG GANGGAATGG TTCTTGCTGG
6201  TNTTCAACTC TGCANGGCTT CCNCTCTGAA TTTCACACGG ANGNCCATT
```

FIGURE 13b (cont.)

```
  1  GCGACTTCTG GTTCCAAAAA GGCGTCTACA GGGAGGTGAC TCCAGCAAGA
 51  AGGGAATTAA CTTTGTCTTC GGGTCACCTG ACAAAGATAA CAAATAAGCA
101  CCATATCAAC GCTTGATCAG AACCGTGTAC CGACGTCCTT GTAATATTCC
151  TGCTATTGCT AGTAGTAGCA ATACTGTCAA ACTGTGCAGA CTTGAGATTC
201  TGGCTTGGAC TTTGCTGAGG TTACCTACTA TATAGAAAGA TAAATAAGAG
251  GTGATGGTGC GGGTCGAGTC CGGCTATATG TGCCAAATAT GCGCCATCCC
301  GAGTCCTCTG TCATAAAGGA
```

FIGURE 14

Genomic clones from *T. tauschii* for SBE-II

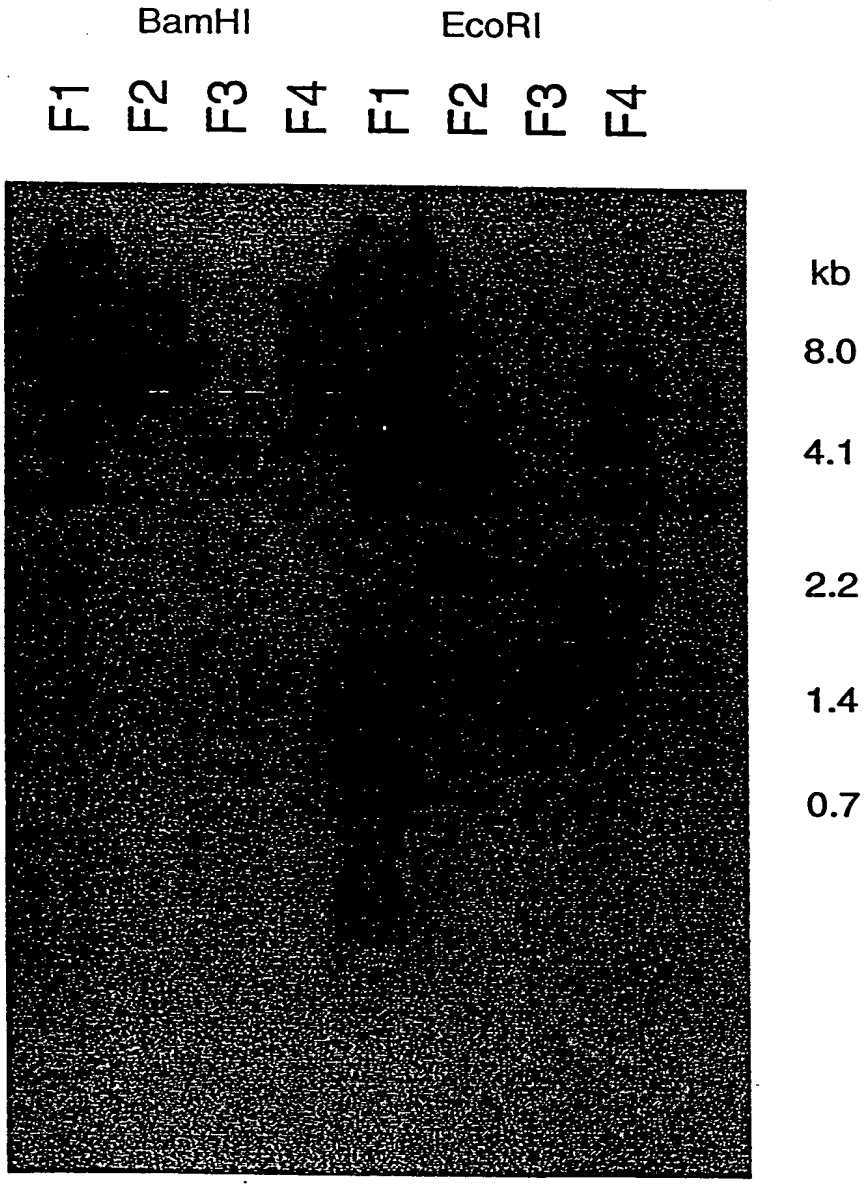


FIGURE 15

N-terminal sequences of cereal starch branching enzymes

Protein	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
	A																					
RICEBEI[B]	A	T	**A**	R	K	N	K	T	**M**	V	T	V	V	E	E	V						
WBE-I_{AD}	V	S	**A**	P	R	**D**	Y	T	**M**	A	T	A	E	D	G	V						
MAIZE BEI[C]	A	T	V	Q	E	**D**	K	T	**M**	A	T	A	K	G	D	V						
RICEBEII[D]	A	A	G	A	S	G	E	-	V	M	I	P	E	G	E	S	D	G	M	P	V	S
WBE-II		A	A	S	P	G	K	-	V	L	V	P	D	G	E	S	D	D	L	A	S	Y
MAIZE BEII[B]	A	A	A	A	A	R	**K**	A	V	**M**	V	**P**	E	G	E	N	D	G	L	A	S	

[A] N-terminal amino acid of the mature polypeptide. [B] Kawasaki *et al.*(1993), [C] Baba *et al.* (1991),

[D] Mizuno *et al.* (1993),[B] Fisher *et al.* (1993)

Residues in the wheat sequences showing identity with the respective maize or rice branching enzyme isoforms are highlighted in bold text.

FIGURE 16a

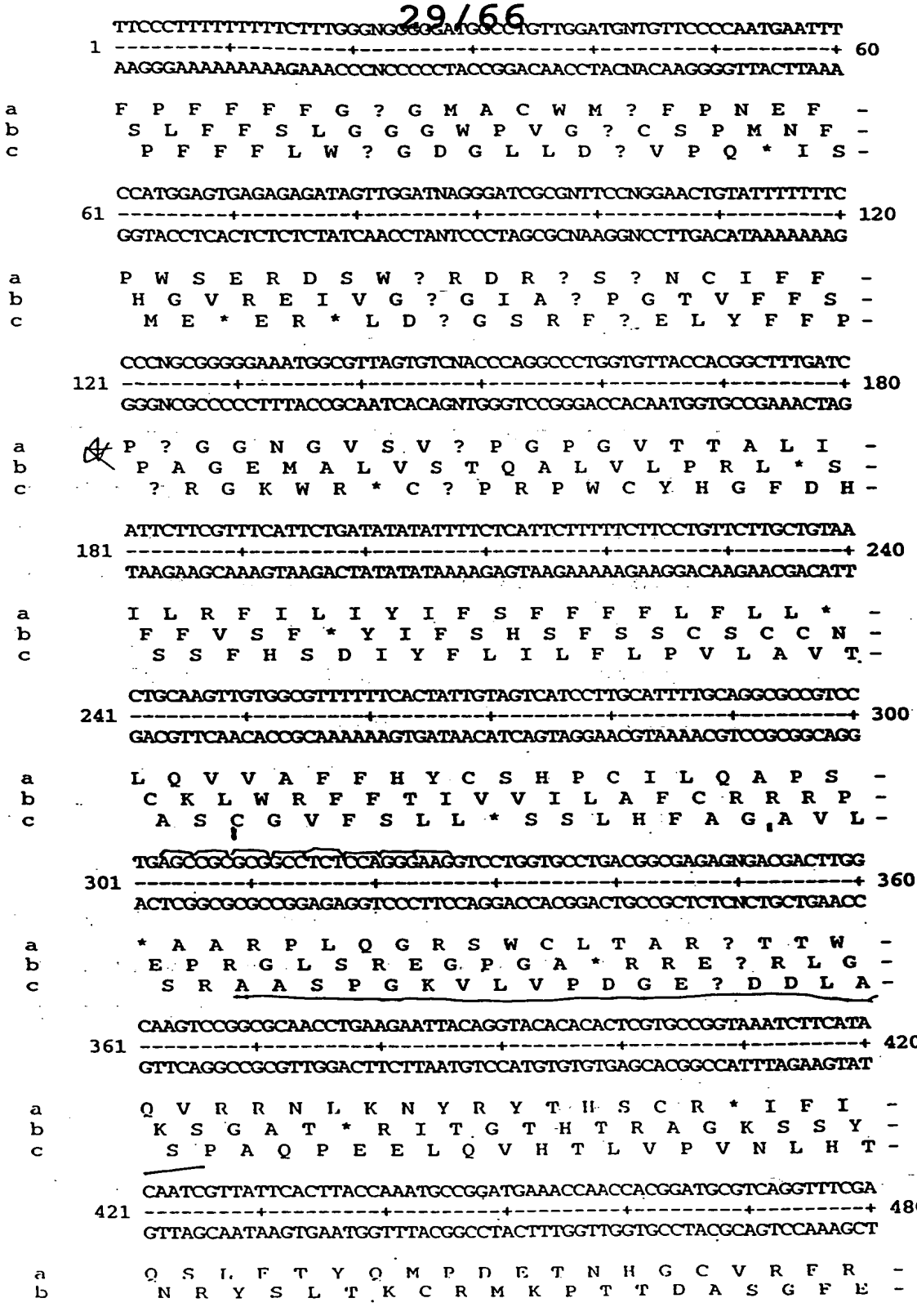


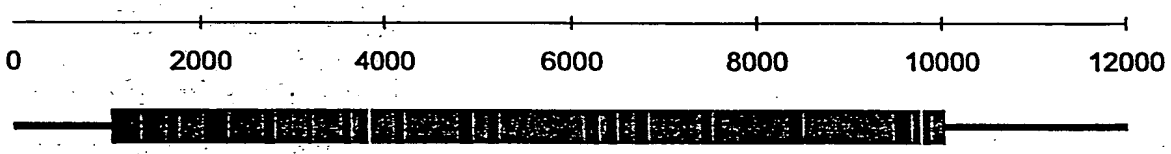
FIGURE 16b

```
  1  MATFAVSGAT LGVARPPAAA QPEELQIPED IEEQTAEVNM TGGTAEKLES
 51  SEPTQGIVET ITDGVTKGVK ELVVGEKPRV VPKPGDGQKI YEIDPTLKDF
101  RSHLDYRYSE YRRIRAAIDQ HEGGLEAFSR GYEKLGFTRS AEGITYREWA
151  PGAHSAALVG DFNNWNPNAD TMTRDDYGVW EIFLPNNADG SPAIPHGSRV
201  KIRMDTPSGV KDSISAWIKF SVQAPGEIPF NGIYYDPPEE EKYVFQHPQP
251  KRPESLRIYE SHIGMSSPEP KINSYANFRD EVLPRIKRLG YNAVQIMAIQ
301  EHSYYASFGY HVTNFFAPSS RFGTPEDLKS LIDRAHELGL LVLMDIVHSH
351  SSNNTLDGLN GFDGTDTHYF HGGPRGHHWM WDSRLFNYGS WEVLRFLLSN
401  ARWWLEEYKF DGFRFDGVTS MMYTHHGLQM TFTGNYGEYF GFATDVDAVV
451  YLMLVNDLIH GLHPDAVSIG EDVSGMPTFC IPVPDGGVGF DYRLHMAVAD
501  KWIELLKQSD ESWKMGDIVH TLTNRRWLEK CVTYAESHDQ ALVGDKTIAF
551  WLMDKDMYDF MALDRPSTPR IDRGIALHKM IRLVTMGLGG EGYLNFMGNE
601  FGHPEWIDFP RGPQTLPTGK VLPGNNNSYD KCRRRFDLGD ADFLRYHGMQ
651  EFDQAMQHLE EKYGFMTSEH QYVSRKHEED KVIIFERGDL VFVFNFHWSN
701  SFFDYRVGCS RPGKYKVALD SDDALFGGFS RLDHDVDYFT TEHPHDNRPR
751  SFSVYTPSRT AVVYALTE*
```

FIGURE 16C

Branching Enzyme-II Genes

Intron/Exon structure of wheat BE-II



Schematic Diagram of a cDNA for BE-II

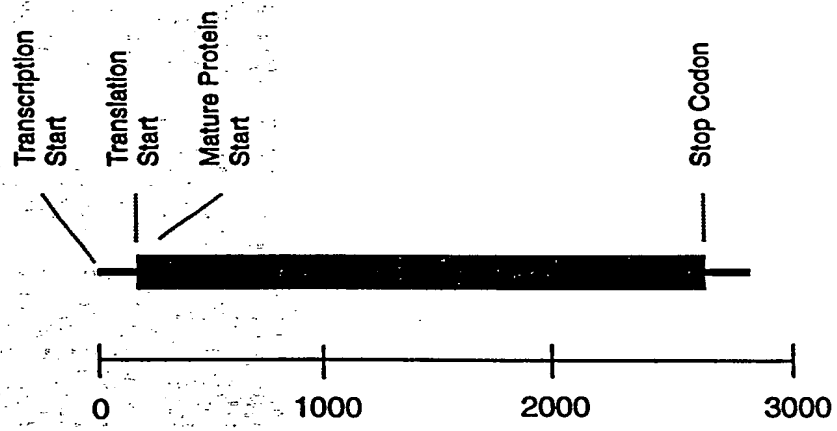


FIGURE 17

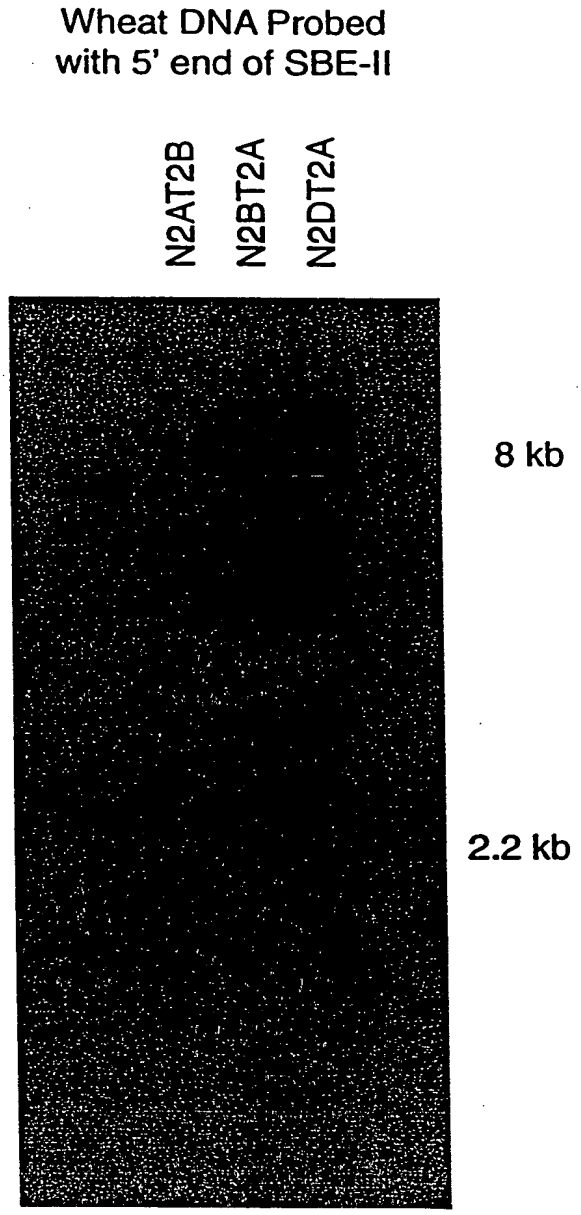

Wheat DNA Probed
with 5' end of SBE-II
N2AT2B
N2BT2A
N2DT2A
8 kb
2.2 kb

FIGURE 18

```
   1  AGAAACACCT CCATTTTAGA TTTTTTTTTT GTTCTTTTCG GACGGTGGGT
  51  CGTGGAGAGA TTAGCGTCTA GTTTTCTTAA AAGAACAGGC CATTTAGGCC
 101  CTGCTTTACA AAAGGCTCAA CCAGTCCAAA ACGTCTGCTA GGATCACCAG
 151  CTGCAAAGTT AAGCGCGAGA CCACCAAAAC AGGCGCATTC GAACTGGACA
 201  GACGCTCACG CAGGAGCCCA GCACCACAGG CTTGAGCCTG ACAGCGGACG
 251  TGAGTGCGTG ACACATGGGG TCATCTATGG GCGTCGGAGC AAGGAAGAGA
 301  GACGCACATG AACACCATGA TGATGCTATC AGGCCTGATG GAAGGAGCAA
 351  CCATGCACCT TTTCCCCTCT GGAAATTCAT AGCTCACACT TTTTTTTAAT
 401  GGAAGCAAGA GTTGGCAAAC ACATGCATTT TCAAACAAGG GAAAATTAAT
 451  TCTCAAACCA CCATGACATG CAATTCTCAA ACCATGCACC GACGAGTCCA
 501  TGCGAGGTGG AAACGAAGAA CTGAAAATCA ACATCCCAGT TGTCGAGTCG
 551  AGAAGAGGAT GACACTGAAA GTATGCGTAT TACGATTTCA TTTACATACA
 601  TGTACAAATA CATAATGTAC CCTACAATTT GTTTTTTGGA GCAGAGTGGT
 651  GTGGTCTTTT TTTTTTACAC GAAAATGCCA TAGCTGGCCC GCATGCGTGC
 701  AGATCGGATG ATCGGTCGGA GACGACGGAC AATCAGACAC TCACCAACTG
 751  CTTTTGTCTG GGANACAATA AATGTTTTTT GTAAACAAAA TAAATACTTA
 801  TAAACGAAGG GTACTAGAGG CCGCTAACGG CATGGCCAGG TAAACGCGCT
 851  CCCAGCCGTT GGTTTGCNAT CTCGTCCTCC CGCACGCAGC GTCGCCTCCA
 901  CCGTCCGTCC GTCGCTGCCA CCTCTGCTGT GCGCGCGCAC AAGGGAGGAA
 951  AACAACGCCG CACACACACT CACACACGGN ACACTCCCCG TGGGTCCCCT
1001  TTCCGGCTTG GCNTCTATCT CCTCTCCCCC GCCCATCCCC ATGCACTGCA
1051  CCGTACCCGC CAGCTTCCAC CCCCGCCGCA CACNTTGCTC CCCCTTCTCA
1101  TCGCTTCTCA ATTAATATCT CCATCACTCG GGTTCCGCGC TGCATTTCGG
1151  CCGGCGGGTT GAGTGAGATC TGGGCGACTG GCTGACTCAA TCACTACGCG
1201  GGGATG
```

FIGURE 19a

```
  1  CCGGCGGGTT GAGTGAGATC TGGGCGACTG GCTGACTCAA TCACTACGCG
 51  GGGATGGCGA CGTTCGCGGT GTCCGGCGCG ACTnTCGGTG TGGCGCGGGC
101  CGGCGTCGGA GTGGCGCGGG CCGGCTCGGA GCGGAGGGGC GGGGCGGACT
151  TGCCGTCGCT GCTCCTCACG AAGAAGGACT CCTCTCGTAC GCCTCGCTCT
201  CTCGAATCTC CCCCGTCTGG CTTTGGCTCC CCTTCTCTCT CCTCTGCGCG
251  CGCATGGCCT GTTCGATGCT GTTCCCCAAT TGATCTCCAT GAGTGAGAGA
301  GATAGCTGGA TTAGGCGATC GCGCTTCCTG AACCTGTATT TTTTCCCCCG
351  CGGGGAAATG CGTTAGTGTC ACCCAGGCCC TGGTGTTACC ACGGCTTTGA
401  TCATTCCTCG TTTCATTCTG ATATATATTT TCTCATTCTT TTTCTTCCTG
451  TTCTTGCTGT AACTGCAAGT TGTGGCGTTT TTTCACTATT GTAGTCATCC
501  TTGCATTTTG CAGGCGCCGT CCTGAGCCGC GCGGCCTCTC CAGGGAAGGT
551  CCTGGTGCCT GACGGCGAGA GnGACGACTT GGCAAGTCCG GCGCAACCTG
601  AAGAATTACA GGTACACACA CTCGTGCCGG TAAATCTTCA TACAATCGTT
651  ATTCACTTAC CAAATGCCGG ATGAAACCAA CCACGGATGC GTCAGGTTTC
701  GAGCTTCTTC TATCAGCATT GTGCAGTACT GCACTGCCTT GTTCATTTTG
751  TTAGCCTTGG CCCCGTGCTG GCTCTTGGGC CACTGAAAAA ATCAGATGGA
801  TGTGCATTCT AGCAAGAACT TCACAACATA ATGCACCGTT TGGGGTTTCG
851  TCAGTCTGCT CTACAATTGC TATTTTTCGT GCTGTAGATA CCTGAAGATA
901  TCGAGGAGCA AACGGCGGAA GTGAACATGA CAGGGGGGAC TGCAGAGAAA
951  CTTCAATCTT CAGAACCGAC TCAGGGCATT GTGGAAACAA TCACTGATGG
```

FIGURE 19b

1001 TGTAACCAAA GGAGTTAAGG AACTAGTCGT GGGGGAGAAA CCGCGAGTTG
1051 TCCCAAAACC AGGAGATGGG CAGAAAATAT ACGAGATTGA CCCAACACTG
1101 AAAGATTTTC GGAGCCATCT TGACTACCGG TAATGCCTAC CCGCTGCTTT
1151 CGCTCATTTT GAATTAAGGT CCTTTCATCA TGCAAATTTG GGGAACATCA
1201 AAGAGACAAA GACTAGGGAC CACCATTTCA TACAGATCCC TTCGTGGTCT
1251 GAGAATATGC TGGGAAGTAA ATGTATAATT GATGGCTACA ATTTGCTCAA
1301 AATTGCAATA CGAATAACTG TCTCCGATCA TTACAATTAA AGAGTGGCAA
1351 ACTGATGAAA ATGTGGTGGA TGGGTTATAG ATTTTACTTT GCTAATTCCT
1401 CTACCAAATT CCTAGGGGGG AAATCTACCA GTTGGGAAAC TTAGTTTCTT
1451 ATCTTTGTGG CCTTTTTGTT TTGGGGAAAA CACATTGCTA AATTCGAATG
1501 ATTTTGGGTA TACCTCGGTG GATTCAACAG ATACAGCGAA TACAAGAGTG
1551 CTGCTATTGA CCAACATGAA GGTGGATTGG AAGCATTTTC TCGTGGTTAT
1601 GAAAAGCTTG GATTTACCCG CAGGTAAATT TAAAGCTTTA TTATTATGAA
1651 ACGCCTCCAC TAGTCTAATT GCATATCTTA TAAGAAAATT TATAATTCCT
1701 GTTTTCCCCT CTCTTTTTTC CAGTGCTGAA GGTATCGTCT AATTGCATAT
1751 CTTATAAGAA AATTTATATT CCTGTTTTCC CCTATTTTCC AGTGCTGAAG
1801 GTATCACTTA CCGAGAATGG GCTCCCTGGA GCGCATGTTA TGTTCTTTTA
1851 AGTTCCTTAA CGAGACACCT TCCAATTTAT TGTTAATGGT CACTATTCAC
1901 CAACTAGCTT ACTGGACTTA CAAATTAGCT TACTGAATAC TGACCAGTTA
1951 CTATAAATTT ATGATCTGGC TTTTGCACCC TGTTACAGTC TGCAGCATTA

FIGURE 19b (cont.)

2001 GTAGGTGACT TCAACAATTG GAATCCAAAT GCAGATACTA TGACCAGAGT

2051 ATGTCTACAG CTTGGCAATT TTCCACCTTT GCTTCATAAC TACTGATACA

2101 TCTATTTGTA TTTATTTAGC TGTTTGCACA TTCCTTAAAG TTGAGCCTCA

2151 ACTACATCAT ATCAAAATGG TATAATTTGT CAGTGTCTTA AGCTTCAGCC

2201 CAAACATTCT ACTGAATTTA GTCCATCTTT TTGAGATTGA AAATGAGTAT

2251 ATTAAGGATG AATGAATACG TGCAACACTC CCATCTGCAT TATGTGTGCT

2301 TTTCCATCTA CAATGAGCAT ATTTCCATGC TATCAGTGAA GGTTTGCTCC

2351 TATTGATGCA GATATTTGAT ATGGTCTTTT CAGGATGATT ATGGTGTTTG

2401 GGAGATTTTC CTCCCTAACA ACGCTGATGG ATCCTCAGCT ATTCCTCATG

2451 GCTCACGTGT AAAGGTAAGC TGGCCAATTA TTTAGTCGAG GATGTAGCAT

2501 TTTCGAACTC TGCCTACTAA GGGTCCCTTT TCCTCTCTGT TTTTTAGATA

2551 CGGATGGATA CTCCATCCGG TGTGAAGGAT TCAATTTCTG CTTGGATCAA

2601 GTTCTCTGTG CAGGCTCCAG GTGAAATACC TTTCAATGGC ATATATTATG

2651 ATCCACCTGA AGAGGTAAGT ATCGATCTAC ATTACATTAT TAAATGAAAT

2701 TTCCAGTGTT ACAGTTTTTT AATACCCACT TCTTACTGAC ATGTGAGTCA

2751 AGACAATACT TTTGAATTTG GAAGTGACAT ATGCATTAAT TCACCTTCTA

2801 AGGGCTAAGG GGCAACCAAC CTTGGTGATG TGTGTATGCT TGTGTGTGAC

2851 ATAAGATCTT ATAGCTCTTT TATGTGTTCT CTGTTGGTTA GGATATTCCA

2901 TTTTGGCCTT TTGTGACCAT TTACTAAGGA TATTTACATG CAAATGCAGG

2951 AGAAGTATGT CTTCCAACAT CTCAACTAAA CGACCAGAGT CACTAAGGAT

FIGURE 19b (cont.)

3001 TTATGAATCA CACATTGGAA TGAGCAGCCC GGTATGTCAA TAAGTTATTT

3051 CACCTGTTTC TGGTCTGATG GTTTATTCTA TGGATTTTCT AGTTCTGTTA

3101 TGTACTGTTA ACATATTACA TGGTGCATTC ACTTGACAAC CTCGATTTTA

3151 TTTTCTAATG TCTTCATATT GGCAAGTGCA AAACTTTGCT TCCTCTTTGT

3201 CTGCTTGTTC TTTTGTCTTC TGTAAGATTT CCATTGCATT TGGAGGCAGT

3251 GGGCATGTGA AAGTCATATC TATTTTTTTT TTGTCAGAGC ATAGTTATAT

3301 ATTGTTGTTG CAATAGCTCG GTATAATGTA ACCATGTTAC TAGCTTAAGA

3351 TTTCCCACTT AGGATGTAAG AAATATTGCA TTGGAGCGTC TCCAGCAAGC

3401 CATTTCCTAC CTTATTAATG AGAGAGAGAC AAGGGGGGGG GGGGGGGGGG

3451 GGTTCCCTTC ATTATTCTGC GAGCGATTCA AAAACTTCCA TTGTTCTGAG

3501 GTGTACGTAC TGCAGGGATC TCCCATTATG AAGAGGATAT AGTTAATTCT

3551 TTGTAACCTA CTTGGAAACT TGAGTCTTGA GGCATCGCTA ATATATACTA

3601 TCATCACAAT ACTTAGAGGA TGCATCTGAA nATTTTAGTG TGATCTTGCA

3651 CAGGAACCGA AGATAAATTC ATATGCTAAT TTTAGGGATG AGGTGTTGCC

3701 AAGAATTAAA AGGCTTGGAT ACAATGCAGT GCAGATAATG GCAATCCAGG

3751 AGCATTCATA CTATGCAAGC TTTGGGTATT CACACAATCC ATTTTTTTCT

3801 GTATACACnT CTTCACCCAT TTGGAGCTAT TACATCCTAA TGCTTCATGC

3851 ACATAAAATA TTTGGATATA ATCCTTTATT AGATATATAG TACAACTACA

3901 CTTAGTATTC TGAnnAAnAA GATCATTTTA TTGTTGTTGG CTTGTTCCAG

3951 GTACCATGTT ACTAATTTTT TTGCACCAAG TAGCCGTTTT GGAACTCCAG

FIGURE 19b (cont.)

```
4001  AGGACTTAAA ATCCTTGATC GATAGAGCAC ATGAGCTTGG TTTGCTTGTT
4051  CTTATGGATA TTGTTCATAG GTAATTAGTC CAATTTAATT TTAGCTGTTT
4101  TACTGTTTAT CTGGTATTCT AAAGGGAAAT TCAGGCAATT ATGATACATT
4151  GTCAAAAGCT AAGAGTGGCG AAAGTGAAAT GTCAAAATCT AGAGTGGCAT
4201  AAGGAAAATT GGCAAAAACT AGAGTGGCAA AAATAAAATT TTCCCATCCT
4251  AAATGGCAGG GCCCTATCGC CGAATATTTT TCCATTCTAT ATAATTGTGC
4301  TACGTGACTT CTTTTTTCTC AGATGTATTA AACCAGTTGG ACATGAAATG
4351  TATTTGGTAC ATGTAGTAAA CTGACAGTTC CATAGAATAT CGTTTTGTAA
4401  TGGCAACACA ATTTGATGCC ATAGATGTGG ATTGAGAAGT TCAGATGCTA
4451  TCAATAGAAT TAATCAACTG GCCATGTACT CGTGGCACTA CATATAGTTT
4501  GCAAGTTGGA AAACTGACAG CAATACCTCA CTGATAAGTG GCCAGGCCCC
4551  ATTTGAACAT ATTACTTAAA GTTCTTCATT TGTCCTAAGT CAAACTTCTT
4601  TAAGTTTGAC CAAGTCTATT GGAAAATATA TCAACATCTA CAACACCAAA
4651  TTACTTTGAT CAGATTAACA ATTTTTATTT TATTATATTA GCACATCTTT
4701  GATGTTGTAG ATATCAGCAC ATTTTTCTAT AGACTTGGTC AAATATAGAG
4751  AAGTTTGACT TAGGACAAAT CTAGAACTTC AATCAATTTG GATCAGAGGG
4801  AACATCAAAT AATATAGATA GATGTCAACA CTTCAACAAA AAAATCAGAC
4851  CTTGTCACCA TATATGCATC AGACCATCTG TTTGCTTTAG CCACTTGCTT
4901  TCATATTTAT GTGTTTGTAC CTAATCTACT TTTCCTTCTA CTTGGTTTGG
4951  TTGATTCTAT TTCAGTTGCA TTGCTTCATC AATGATTTTG TGTACCCTGC
```

FIGURE 19b (cont.)

```
5001  AGTCATTCGT CAAATAATAC CCTTGACGGT TTGAATGGTT TCGATGGCAC
5051  TGATACACAT TACTTCCACG GTGGTCCACG CGGCCATCAT TGGATGTGGG
5101  ATTCTCGTCT ATTCAACTAT GGGAGTTGGG AAGTATGTAG CTCTGACTTC
5151  TGTCACCATA TTTGGCTAAC TGTTCCTGTT AATCTGTTCT TACACATGTT
5201  GATATTCTAT TCTTATGCAG GTATTGAGAT TCTTACTGTC AAACGCGAGA
5251  TGGTGGCTTG AAGAATATAA GTTTGATGGA TTTCGATTTG ATGGGGTGAC
5301  CTCCATGATG TATACTCACC ATGGATTACA AGTAAGTCAT CAAGTGGTTT
5351  CAGTAACTTT TTTAGGGCAC TGAAACAATT GCTATGCATC ATAACATGTA
5401  TCATGATCAG GACTTGTGCT ACGGAGTCTT AGATAGTTCC CTAGTATGCT
5451  TGTACAATTT TACCTGATGA GATCATGGAA GATTGGAAGT GATTATTATT
5501  TATTTTCTTT CTAAGTTTGT TTCTTGTTCT AGATGACATT TACTGGGAAC
5551  TATGGCGAAT ATTTTGGATT TGCTACTGAT GTTGATGCGG TAGTTTACTT
5601  GATGCTGGTC AACGATCTAA TTCATGGACT TTATCCTGAT GCTGTATCCA
5651  TTGGTGAAGA TGTAAGTGCT TACAGTATTT ATGATTTTTA ACTAGTTAAG
5701  TAGTTTTATT TTGGGGATCA GTCTGTTACA CTTTTTGTTA GGGGTAAAAT
5751  CTCTCTTTTC ATAACAATGC TAATTTATAC CTTGTATGAT AATGCATCAC
5801  TTAnGTAATT TGAAAAGTGC AAGGGCATTC AAGCTTACGA GCATATTTTT
5851  TGATGGCTGT AATTTATTTG ATAGTATGCT TGTTTGGGTT TTTCAATAAG
5901  TGGGAGTGTG TGACTAATGT TGTATTATTT ATTTAATTGC GGAAGAAATG
5951  GGCAACCTTG TCAATTGCTT CAGAAGGCTA ACTTTGATTC CATAAACGCT
```

FIGURE 19b (cont.)

```
6001  TTGGAAATGA GAGGCTATTC CCAAGGACAT GAATTATACT TCAGTGTGTT
6051  CTGTACATGT ATTTGTAATA GTGGTTTAAC TTAAATTCCT GCACTGCTAT
6101  GGAATCTCAC TGTATGTTGT nAGTGTACAC ATCCACAAAC AAGTAATCCT
6151  GAGCTTTCAA CTCATGAGAA AATAnGAnGT CCGCTTCTGC CAGCATTAAC
6201  TGTTCACAGT TCTAATTTGT GTAACTGTGA AATTGTTCAG GTCAGTGGAA
6251  TGCCTACATT TTGCATCCCT GTTCCAGATG GTGGTGTTGG TTTTGACTAC
6301  CGCCTGCATA TGGCTGTAGC AGATAAATGG ATTGAACTCC TCAAGTAAGT
6351  GCAGGAATAT TGGTGATTAC ATGCGCACAA TGATCTAGAT TACATTTTCT
6401  AAATGGTAAA AAGGAAAATA TGTATGTGAA TATCTAGACA TTTGCCTGTT
6451  ATCAGCTTGA ATACGAGAAG TCAAATACAT GATTTAAATA GCAAATCTCG
6501  GAAATGTAAT GGCTAGTGTC TTTATGCTGG GCAGTGTACA TTGCGCTGTA
6551  GCAGGCCAGT CAACACAGTT AGCAATATTT TCAGAAACAA TATTATTTAT
6601  ATCCGTATAT GAnGAAAGTT AGTATATAAA CTGTGGTCAT TAATTGTGTT
6651  CACCTTTTGT CCTGTTTAAG GATGGGCAGT AGGTAATAAA TTTAGCCAGA
6701  TAAAATAAAT CGTTATTAGG TTTACAAAAG GAATATACAG GGTCATGTAG
6751  CATATCTAGT TGTAATTAAT GAAAAGGCTG ACAAAAGGCT CGGTAAAAAA
6801  AACTTTATGA TGATCCAGAT AGATATGCAG GAACGCGACT AAAGCTCAAA
6851  TACTTATTGC TACTACACAG CTGCCAATCT GTCATGATCT GTGTTCTGCT
6901  TTGTGCTATT TAGATTTAAA TACTAACTCG ATACATTGGC AATAATAAAC
6951  TTAACTATTC AACCAATTTG GTGGATACCA GAnATTTCTG CCCTCTTGTT
```

FIGURE 19b (cont.)

```
7001  AGTAATGATG TGCTCCCTGC TGCTGTTCTC TGCCGTTACA AAAGCTGTTT
7051  TCAGTTTTTT GCATCATTAT TTTTGTGTGT GAGTAGTTTA AGCATGTTTT
7101  TTGAAGCTGT GAGCTGTTGG TACTTAATAC ATTCTTGGAA GTGTCCAAAT
7151  ATGCTGCAGT GTAATTTAGC ATTTCTTTAA CACAGGCAAA GTGACGAATC
7201  TTGGAAAATG GGCGATATTG TGCACACCCT AACAAATAGA AGGTGGCTTG
7251  AGAAGTGTGT AACTTATGCA GAAAGTCATG ATCAAGCACT AGTTGGTGAC
7301  AAGACTATTG CATTCTGGTT GATGGATAAG GTACTAGCTG TTACTTTTGG
7351  ACAAAAGAAT TACTCCCTCC CGTTCCTAAA TATAAGTCTT TGTAGAGATT
7401  CCACTATGGA CCACATAGTA TATAGATGCA TTTTAGAGTG TAGATTCACT
7451  CATTTTGCTT CGTATGTAGT CCATAGTGAA ATCTCTACAG AGACTTATAT
7501  TTAGGAACGG AGGGAGTACA TAATTGATTT GTCTCATCAG ATTGCTAGTG
7551  TTTTCTTGTG ATAAAGATTG GCTGCCTCAC CCATCACCAG CTATTTCCCA
7601  ACTGTTACTT GAGCAGAATT TGCTGAAAAC GTACCATGTG GTACTGTGGC
7651  GGCTTGTGAA CTTTGACAGT TATGTTGCAA TTTTCTGTTC TTATTTATTT
7701  GATTGCTTAT GTTACCGTTC ATTTGCTCAT TCCTTTCCGA GACCAGCCAA
7751  AGTCACGTGT TAGCTGTGTG ATCTGTTATC TGAATCTTGA GCAAATTTTA
7801  TTAATAGGCT AAAATCCAAC GAATTATTTG CTTGAATTTA AATATACAGA
7851  CGTATAGTCA CCTGGCTCTT TCTTAGATGA TTACCATAGT GCCTGAAGGC
7901  TGAAATAGTT TTGGTGTTTC TTGGATGCCG CCTAAAGGAG TGATTTTTAT
7951  TGGATAGATT CCTGGCCGAG TCTTCGTTAC AACATAACAT TTTGGAGATA
```

FIGURE 19b (cont.)

8001 TGCTTAGTAA CAGCTCTGGG AAGTTTGGTC ACAAGTCTGC ATCTACACGC

8051 TCCTTGAGGT TTTATTATGG CGCCATCTTT GTAACTAGTG GCACCTGTAA

8101 GGAAACACAT TCAAAAGGAA ACGGTCACAT CATTCTAATC AGGACCACCA

8151 TACTAAGAGC AAGATTCTGT TCCAATTTTA TGAGTTTTTG GGACTCCAAA

8201 GGGAACAAAA GTGTCTCATA TTGTGCTTAT AACTACAGTT GTTTTTATAC

8251 CAGTGTAGTT TTATTCCAGG ACAGTTGATA CTTGGTACTG TGCTGTAAAT

8301 TATTTATCCG ACATAGAACA GCATGAACAT ATCAAGCTCT CTTTGTGCAG

8351 GATATGTATG ATTTCATGGC TCTGGATAGG CTTCAACTCT TCGCATTGAT

8401 CGTGGCATAG CATTACATAA AATGATCAGG CTTGTCACCA TGGGTTTAGG

8451 TGGTGAAGGC TATCTTAACT TCATGGGAAA TGAGTTTGGG CATCCTGGTC

8501 AGTCTTTACA ACATTATTGC ATTCTGCATG ATTGTGATTT ACTGTAATTT

8551 GAACCATGCT TTTCTTTCAC ATTGTATGTA TTATGTAATC TGTTGCTTCC

8601 AAGGAGGAAG TTAACTTCTA TTTACTTGGC AGAATGGATA GATTTTCCAA

8651 GAGGCCCACA AACTCTTCCA ACCGGCAAAG TTCTCCCCTG GAAATAACAA

8701 TAGTTATGAT AAATGCCGCC GTAGATTTGA TCTTGTAAGT TTTAGCTGTG

8751 CTATTACATT CCCTCACTAG ATCG

FIGURE 19b (cont.)

COMPARISON OF N-TERMINAL SEQUENCES
OF SOLUBLE STARCH SYNTHASE

GRYVAELSREGPAARP Deduced from wheat cDNA

GPYVAELSPEGPAAPP Wheat N-terminal

FIGURE 20a

```
   1  TCTCCCACTC TTCTCTCCCC GCGCACACCG AGTCGGCACC GGCTCATCAC
  51  CCATCACCTC GGCCTCGGCC ACCGGCAAAC CCCCCGATCC GCTTTTGCAG
 101  GCAGCGCACT AAAACCCCGG GGAGCGCGCC CCGCGGCAGC AGCAGCACCG
 151  CAGTGGGAGA GAGAGGCTTC GCCCCGGCCC GCACCGAGCG GGGCGATCCA
 201  CCGTCCGTGC GTCCGCACCT CCTCCGCCTC CTCCCCTGTC CCGCGCGCCC
 251  ACACCCATGG CGGCGACGGG CGTCGGCGCC GGGTGCCTCG CCCCCAGCGT
 301  CCGCCTGCGC GCCGATCCGG CGACGGCGGC CCGGGCGTCC GCCTGCGTCG
 351  TCCGCGCGCG GCTCCGGCGC TTGGCGCGGG GCCGCTACGT TGCCGAGCTC
 401  AGCAGGGAGG GCCCCGCGGC GCGCCCCGCG CAGCAGCAGC AACTGGCCCC
 451  GCCGCTCGTG CCAGGCTTCC TCGCGCCGCC GCCGCCCGCG CCCGCCCAGT
 501  CGCCGGCCCC GACGCAGCCG CCCCTGCCGG ACGCCGGCGT GGGGGAACTC
 551  GCGCCCGACC TCCTGCTCGA AGGGATTGCT GAGGATTCCA TCGACAGCAT
 601  AATTGTGGCT GCAAGTGAGC AGGATTCTGA GATCATGGAT GCGAATGAGC
 651  AACCTCAAGC TAAAGTTACA CGTAGCATCG TGTTTGTGAC TGGTGAAGCT
 701  GCTCCTTATG CAAAGTCAGG GGGGCTGGGA GATGTTTGTG GTTCGTTACC
 751  AATTGCTCTT GCTGCTCGTG GTCACCGTGT GATGGTTGTA ATGCCAAGAT
 801  ACTTGAATGG GTCCTCTGAT AAAAACTATG CAAAGGCATT ATACACTGGG
 851  AAGCACATTA AGATTCCATG CTTTGGGGGA TCACATGAAG TGACCTTTTT
 901  TCATGAGTAT AGAGACAACG TCGATTGGGT GTTTGTCGAT CATCCGTCAT
 951  ATCATAGACC AGGAAGTTTA TATGGAGATA ATTTTGGTGC TTTTGGTGAT
```

FIGURE 20b

1001 AATCAGTTCA GATACACACT CCTTTGCTAT GCTGCATGCG AGGCCCCACT

1051 AATCCTTGAA TTGGGAGGAT ATATTTATGG ACAGAATTGC ATGTTTGTTG

1101 TGAACGATTG GCATGCCAGC CTTGTGCCAG TCCTTCTTGC TGCAAAATAT

1151 AGACCATACG GTGTTTACAG AGATTCCCGC AGCACCCTTG TTATACATAA

1201 TTTAGCACAT CAGGGTCTGG AGCCTGCAAG TACATATCCT GATCTGGGAT

1251 TGCCAccTGA ATGGTATGGA GCTTTAGAAT GGGTATTTCC AGAATGGGCA

1301 AGGAGGCATG CCCTTGACAA GGGTGAGGCA GTTAACTTTT TGAAAGGAGC

1351 AGTCGTGACA GCAGATCGAA TTGTGACCGT CAGTCAGGGT TATTCATGGG

1401 AGGTCACAAC TGCTGAAGGT GGACAGGGCC TCAATGAGCT CTTAAGCTCC

1451 CGAAAAAGTG TATTGAATGG AATTGTAAAT GGAATTGACA TTAATGATTG

1501 GAACCCCACC ACAGACAAGT GTCTCCCTCA TCATTATTCT GTCGATGACC

1551 TCTCTGGAAA GGCCAAATGT AAAGCTGAAT TGCAGAAGGA GCTGGGTTTA

1601 CCTGTAAGGG AGGATGTTCC TCTGATTGGC TTTATTGGAA GACTGGATTA

1651 CCAGAAAGGC ATTGATCTCA TTAAAATGGC CATTCCAGAG CTCATGAGGG

1701 AGGACGTGCA GTTTGTCATG CTTGGATCTG GGGATCCAAT TTTTGAAGGC

1751 TGGATGAGAT CTACCGAGTC GAGTTACAAG GATAAATTCC GTGGATGGGT

1801 TGGATTTAGT GTTCCAGTTT CCCACAGAAT AACTGCAGGT TGCGATATAT

1851 TGTTAATGCC ATCCAGGTTT GAACCTTGTG GTCTTAATCA GCTATATGCT

1901 ATGCAATATG GTACAGTTCC TGTAGTTCAT GGAACTGGGG GCCTCCGAGA

1951 CACAGTCGAG ACCTTCAACC CTTTTGGTGC AAAAGGAGAG GAGGGTACAG

FIGURE 20b (cont.)

```
2001  GGTGGGCGTT CTCACCGCTA ACCGTGGACA AGATGTTGTG GGCATTGCGA
2051  ACCGCGATGT CGACATTCAG GGAGCACAAG CCGTCCTGGG AGGGGCTCAT
2101  GAAGCGAGGC ATGACGAAAG ACCATACGTG GGACCATGCC GCCGAGCAGT
2151  ACGAGCAGAT CTTCGAATGG GCCTTCGTGG ACCAACCCTA CGTCATGTAG
2201  ACGGGGACTG GGGAGGTCGA AGCGCGGGTC TCCTTGAGCT CTGAAGACAT
2251  GTTCCTCATC CTTCCGCGGC CCGGAAGGAT ACCCCTGTAC ATTGCGTTGT
2301  CCTGCTACAG TAGAGTCGCA ATGCGCCTGC TTGCTTGGTC CGCCGGTTCG
2351  AGAGTAGATG ACGGCTGTGC TGCTGCGGCG GTGACAGCTT CGGGTGGATG
2401  ACAGTTACAG TTTTGGGGAA TAAGGAAGGG ATGTGCTGCA GGATGGTTAA
2451  CAGCAAAGCA CCACTCAGAT GGCAGCCTCT CTGTCCGTGT TACAGCTGAA
2501  ATCAGAAACC AACTGGTGAC TCTTTAGCCT TAGCGATTGT GAAGTTTGTT
2551  GCATTCTGTG TATGTTGTCT TGTCCTTAGC TGACAAATAT TAGACCTGTT
2601  GGAGAATTTT ATTTATCTTT GCTGCTGTTG TTTTTGTTTT GTTAAAAAAA
2651  AAAAAAAAAA AA
```

FIGURE 20b (cont.)

```
   1  GAGCTCCGAG AAnAGATTCC TATCATCGTC TTGGTGAGGT GAGGTTATGG
  51  TTTCTTGTCA TGTGGGCAGA TTTGGTGCCA GATGCTTCAT ATCTATTCAA
 101  GGGTTCAGCG GCAACAACTG CGGCTCCAGA GCGATGGTCC TTAAGGGCAC
 151  GTGCACGAAG ACTTCACGGC TGTTATCGAC AAGGTCAAGC CGGCTCCGAT
 201  AGGGGAGCAG CGACAGCGGC GCGTCAACCG CTCGTTCTGG CGGCAGTAGT
 251  GGTCGTTCGG TGCTCTCGGA ACCTCGATGT AATTTTTATG ATTTTAGAGA
 301  TGCTTTGTAc TTCcGATCGa TGAACTCTGA TAATAGATAT CTcTTCTcTc
 351  GCAAAAAAaG aGAGTTTTCA AcTGAAAACA AAaGaGTTTC AcTAGTTCTT
 401  CTTTTAGAAA CAGAGTTTCA cTAGCAcTTT TTTTTGcGAG AAGTcGAGTT
 451  TCAcTAAGTA cTAAaCCCAC GCAaTTATTC TCAAAAAAAA AACCCAcGcA
 501  ACTGTcTGgA TcCATCTTCG TTTTTTCCCC GAGAATCGTC TGgATcCATT
 551  TTCGTGTGCG AgGCATCCTC TCATTTTGcA cGgcCcAGcT cTcTTcTcGC
 601  CGGcGTAcGc TGctAcATgT cGgcAcTCcA cGCAAACAAA AaGAaGCCCA
 651  ACCGAAAAcG cAcGcGCcTT TcCAgGcTCA ccACGGaAAA AAaTACcAcG
 701  cGCcGcTcAC GAgCAAACCG TgACAACAGC CAGCCAGATA TGGCAACGGA
 751  GGcACGGGCC GcACACAGCC AcTGAAAACC GCAGcTGcTC TTCCGTCCGT
 801  CCGTCCcTCC GCCCGTCCGC gCcAcTCCAc TCGCCTTGCC CCAcTCCCAc
 851  TCTTCTCTCC CCGCGCACAC CGAGTCGGCA CCGGCTCATC ACCCATCACy
 901  TCGGCcTCGG CCACCGGCAA ACCCCCCGAT CCGCTTTTGC AGGCAGCGCA
 951  CTAAAACCCC GGGGAGCGCG CCCCGcgg.C AGCAGCAGCA CCGCAGTGGG
1001  AGAGAGAGGC TTCGCCCCGG CCCGCACCGA GCGGGGCGAT CCACCGTCCG
1051  TGCGTCCGCA CCTCCTCCGC CTCCTCCCCT GTCCCGCGCG CCCACACCCA
```

FIGURE 20c

1101 TGGCGGCGAC GGGCGTCGGC GCCGGGTGCC TCGCCCCCAG CGTCCGCcTG

1151 CGCGCCGATC CGGCGACGGC GGCCCGGGCG TCCGCtTGCG TCGTCCGCGC

1201 GCGGCTCCGG CGcTTGGCGC GGGgCCGyTA CGTCGCCGAG CTCaGcAgGG

1251 AGGGCCCCGC GGcGCGCCCC GCGCAGCAGC AGCAACTGGC CCCGCCGCTC

1301 GTGCCAGGCT TCCTCGCGCC GCCGCCGCCC GCGCCCGCCC AGTCGCCGGC

1351 CCCGACGCAG CCGCCCcTGC CGGACgCCGG CgTGGGgGAA CTCGCGCCcG

1401 ACcTCcTGCT CGAAGGTAAA AAACAaggct gaatcCtcAg atcaCtcCGc

1451 gTcttcgTTt taccAaAtac ggtactGcga aGtgGtgcTg TATaTGtgaa

1501 gTtTcTgtcg aTtTcttcct gacggaTgtt cagtcgattc agtTgTATAT

1551 aTGtgAtacg ttcgtTgttc atcgatcgtA cAgaTttacc agCACactAg

1601 atAgAaatcG AgaccgaCGc GggcAgatca AtAgaTTTtT ctagaskTTT

1651 wwTkGrwtCG TGAGATGATT GATTGGGGTG GCGTGTCGAT ACGATAGCGG

1701 TGCACCGCCG ATGTATCGGG GCATGTGCAC GTGGTTGGGT CTCAGCAGAC

1751 ATATCACTAG ACTGGTATCG TAATTTACTA GTACTACTGG AAAGAGGACT

1801 AAAAAGGCTA GGCCAAGTGC ACGCATGTTG GGAACGTTGT TAAATTGATG

1851 AGTTTGTCCT TTGCTTGGGC TGGTATTATT ACCAAAAAAT GGTGTTAGTC

1901 CCTGTACTTA TTAATGGGaA AATCtTAACA TGACACTgGG GTTTATGAGT

1951 CTCCAATTGT ATATTCTCAG CACTCAACTG ATTTTACTGA TACTGTAGTG

2001 GAAATGACAC GTGAGCAcCC CCCTTCAAGG AATGCAATGC TTCTTTCTGT

2051 TTTAtATTAC AGGAACTAGA AGGAGCtTCC ACCTTTGAGT ACAGAAGTAC

2101 TCCCTCCGTT CCAAAATAGA TGACTCAACT TTGTACTAAT TTTGTACTAT

2151 AGTTAGTACA AAGTTGAGTC ATCTATTTTA GAACGGAGGG AGTAGTATCG

FIGURE 20c (cont.)

```
2201  AAATTGAAGA CCCTTGTATT ACTGTCTTGT TTTTCAATGA AAATGGGAGG
2251  CCCATGCAGT AAGTCACATG GGCACCTGGG AGGCTGGGAT CATGTGTGCT
2301  TTGCAGAGTA CTAGACCCAG CTCACCCTCT GTTAGATTAC TTGTTGGGCT
2351  GCTACTTTGT GTTTGCTGTG CAGTATATCA GACATCCTGA ATTTGGCATC
2401  TAGCTGAGAA CAGAATGCAG GTTGCACCAT TCTTATTATT GCTAAACTGT
2451  TGTCACGCAA TTTATAAAGA ATGTGATCTT CTGAGTATTA ATTAATCATG
2501  TTCTGCTAAT ATCTGTCCTC GCTCTGGTGT TGACAAATAT ACCATATGAA
2551  TATTTTCCAT TTTGCAACCA GGGATTGCTG AGGATTCCAT CGACAGCATA
2601  ATCGTGGCTG CAAGTGAGCA GGATTCTGAG ATCATGGATG CGAATGAGCA
2651  ACCTCAAGCT AAAGTTACAC GTAGCATCGT GTTTGTGACT GGTGAAGCTG
2701  CTCCTTATGC AAAGTCAGGG GGGCTGGGAG ATGTTTGTGG TTCGTTACCA
2751  ATTGCTCTTG CTGCTCGTGG TCACCGTGTG ATGGTTGTAA TGCCAAGATA
2801  CTTGAATGGG TCCTCTGATA AAAACTATGC AAAGGCATTA TACACTGCGA
2851  AGCACATTAA GATTCCATGC TTTGGGGGAT CACATGAAGT GACCTTTTTT
2901  CATGAGTATA GAGACAACGT CGATTGGGTG GGTACACAAT CACCTTCTTA
2951  TTCTCTGTTG AATTGTAGCA ACTGTTTATC CTTGTTTACA CTTCTTTTAG
3001  CCCTGCAAAG ACATATGTGA TTTCCATACT TTTTTGTTAT TTCCCTTGTA
3051  CTCTTGCTCA TGAAGGTCAA AATATCATAT ATCCATGGAA GTCATGCATG
3101  TGCCTAGTAT TTTTGGTGTC GGTGCCTTTA ACTTTCAGGG ATTAATACGT
3151  GGAATTTGAT AACTAAAGTT TATTTTATTG AAAAAAATTG TAGGTTGGCT
3201  GAGCCCACAG CCACGCAGTG GCACCACTGC TTGCACATGA TTTTGCATTT
3251  CTGTTTGCAC CGAGCACTTC ATGTGAATAA GGTGTAAAAT CATAAAGTAC
```

FIGURE 20c (cont.)

3301 CAATTTTATT CTGCCAATTG CACTTAAGAG TATATACATT TATCTTGGCC

3351 TCAATCATGG GAGTACTGTG CATTCAGTGC ACCATCATTG TTCTAAGGAG

3401 AAAATGTGGG TGCAAGGAAG ACACTTTTGT CCCTTAATAA AAGGCAGGCA

3451 CTCTGTTGTC ATATAGATAG AAAGCAACAA ACTTATTTCA AAGAGCTAAC

3501 AATGGCAAAA GAACCAAAAA AAGCATGCTA AGGCGGTGAC ACCAAAAGGT

3551 GAGGGGGGCC TTGTGACTGA CAGCACCCCA AACTATTGCC ATTGTTTTAC

3601 TAAATGAAGA TCATTTTAGA AGCTCTCAGG AACTTCGAAA ACAGTGGCTT

3651 TCCGTCCACA GATCGTCTGT TAATATTTTT GTCCAGTGAT ACTTTTTTTG

3701 CTCCTTACAA GAGTGCCTAT GTTGACATAT ACATTGTTAA GTTGTTCATA

3751 AGTTTACTTC TTATTCTAAA CAGCAAGTGC CTAATGCTTG CATTTATTTT

3801 GGCTATTTAT TTTTATTCTC ATTTCAATCA ACACTTTTGT TCAGGTGTTT

3851 GTCGATCATC CGTCATATCA TAGACCAGGA AGTTTATATG GAGATAATTT

3901 TGGTGCTTTT GGTGATAATC AGGTACACTA CACTATACTA AGCTCCTAGT

3951 TGACTAAGTC GTAAGTTGTA CCTCCTCGCT GACCGGCTGC TCTATGTCGT

4001 GCAGTTCAGA TACACACTCC TTTGCTATGC TGCATGCGAg GCCCCACTAA

4051 TCCTTGAATT GGGAGGATAT ATTTATGGAC AGAATTGCAT GTTTGTTGTG

4101 AACGATTGGC ATGCCAGCCT TGTGCCAGTG TACGTTGTTT GTGGATCTGA

4151 AAGTCCAATC CTTTATTCAT TCTCTGCTTT GCAGTGTGCC CATGTCTACA

4201 TTTCTTTTAT GCTTTTTTCA TGTCTGTTCT TATATTGCAT ATATGCTTAT

4251 GGAGTCTAAA AGTTACCGGA GGGAATAACT CtTAAGGAtT TCCTCAATCA

4301 ATTATCtTTA GCtTTAGTTA ACAtTTACTG TGGCAAACAT AATGTGtTTT

4351 GAGAtTTACA ArkTCAGAGA TTgCACtTCA CTAGtTCGTA gCTAAtCyGA

FIGURE 20c (cont.)

```
4401  tGtTTTCCCC GAGaAAATGC CtAAAGCTTT gtGTCtTGAT gCAtTGATAG
4451  aAAAAGAgtT TATGTaCACT CCcaAAGAgG GGACCcaAAA TTaCaACAcC
4501  AcACCCctGA GaACtAgGcG CtGCCgGAAg AAgCGATgCa AGccCCAcTG
4551  CCCCTGCCTT AGCTCAAAGC CGGGCgTCAG cCTTGATTrT GTCAAGTAAG
4601  CTAGCAGTGC TAGATTGCGC AAGGTCGATT CGTCGAAGAT GACAGTGTTG
4651  CGCTGCTTCC AAATCCACCA AACTATGAGC ATGATCACTG GAGAAGTACC
4701  TTTTCTCGCG GCTGAGGGGG TGGACTGGTG GTCTGCTGCT GCCAGTTTTC
4751  AGATAATCTG AAAAATGCAT GTTTTGATGA TTTTAGTATC TTGCGGACCC
4801  TGGGTACCAC CTAAGCTTTC ACACAGTAAT TTGCAGTTAC ACcTATAAAA
4851  GTAACGGTCA TGATATGCAT GTGTTTTGGG TAGATCATGG TGCATGCATT
4901  TTAGGAATTA GGACATGCCA GAACCACGTG AGGCTTATGG GGCAATTCAT
4951  TTGTTCCATT ATACGAGTCA TGAATATGGT TCAGCATGTT TGGACGCTAC
5001  TTGTTTGGGG CAATTTCAGA TGGTGAATTG TAGCTGCTTG ATGTTGGCTA
5051  GCTGGCTTAT TTTGTACAAG TATCGATGTT AGATGCATAT TTCCTTTTGT
5101  TCTTGTGCTG TTTGCCATGT TGTATTCCCC TTTTCTGTCG CCAGTGTTGC
5151  ATGTTAAATT GGTTTTCATT ACATAATCAA CTTTGTTGCT GACATCAGTC
5201  ATTTTTATTC AGCCTTCTTG CTGCAAAATA TAGACCATAC GGTGTTTACA
5251  GAGATTCCCG CAGCACCCTT GTTATACATA ATTTAGCACA TCAGGTTTGG
5301  GTCTATCACC TTTCATTATC CGTACATGGC TTTGTAAGTC GGTTCACACG
5351  TATCGTCATA CTGTATGTTA TTTCAATGTC ATTAgGGTGT GGAGCCTGCA
5401  AGTACATATC CTGATCTGGG ATTGCCACCT GAATGGTATG GAGCTtTAGA
5451  ATGGGTATTT CCAGAATGGG CAAGGAGGCA TGCCCTTGAC AAGGGTGAGG
```

FIGURE 20c (cont.)

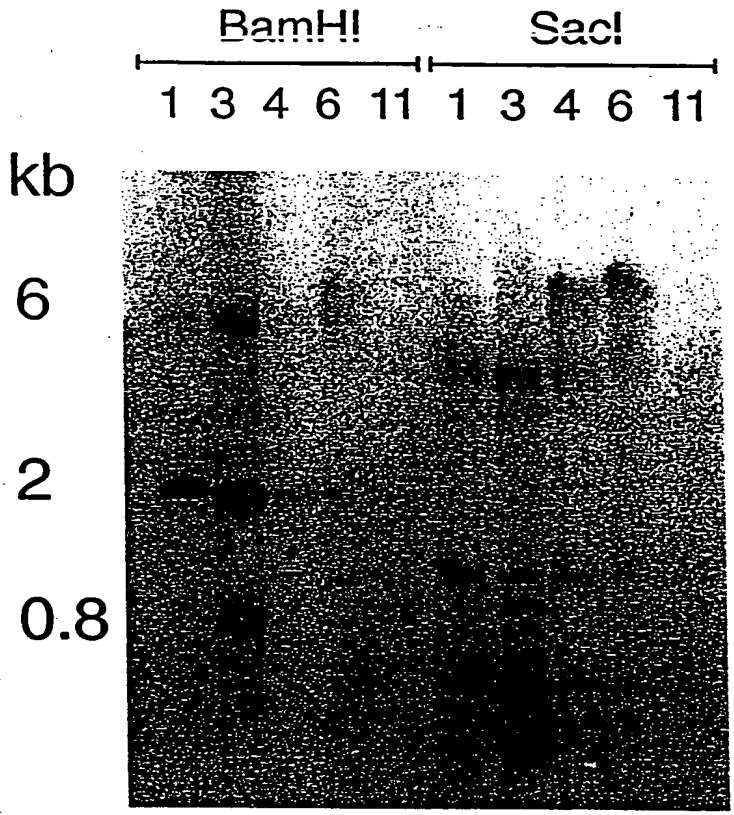
```
5501  CAGTTAACTT TTTGAAAGGA GCAGTTGTGA CAGCAGATCG AATTGTGACC
5551  GTCAGTCAGG TGAAATACTC AATACTTCTC TTTTTTCTTT GCGGGATGTT
5601  CTTCAGTTCA ATTGCCCTGT CTTTCACCCA ATTAAGAAAT GATTTAATCT
5651  TTTGTTTCTA GGGTTATTCA TGGGAGGTCA CAACTGCTGA AGGTGGACAG
5701  GGCcTCAATG AGCTCTTAAG CTCCCGAAAA AGTGTAtTGA ATGGTAACTA
5751  TAtTTGAATC CACTTATCTT C.TTCTGAAA CATATTTACA GAAATAGATG
5801  GATGGGTTGC AAGAATAAAT TCAGTTTGCT CTTTCGGTAT GAAGGAATTG
5851  TAAATGGAAT TGACATTAAT GATTGGAACC CCACCACAGA CAAGTGTCTC
5901  CCTCATCATT ATTCTGTCGA TGACCTCTCT GGAAAGGTGT GTGGATAGTA
5951  CCcTATATAA TAACATGTAT ATCTGATC.T AGTACTTTCT TTTTCTTTGC
6001  TAGTTTGCTT CCCATGATGT TCTCACTAAC TAATCCTATG TGGTTTGGCA
6051  TACTTGTCAG GCCAAATGTA AAGCTGAATT GCAGAAGGAG CTGGGTTTAC
6101  CTGTAAGGGA gGATGTTCcT CTGGTTaGAT ACAAACCCcT aAGATATaTA
6151  TtTtTTAAAT CCCTAAAAAA AAcTTGCCGA TCATCTCaTT AGCTTGATTC
6201  ACAGATTGGC TtTATTGGAA GACTGGATTA CCAGAAAGGC ATTGATCTCA
6251  TTAAAATGGC CATTCCAGAG CTC
```

FIGURE 20c (cont.)

```
  1  MAATGVGAGC LAPSVRLRAD PATAARASAC VVRARLRRLA RGRYVAELSR
 51  EGPAARPAQQ QQLAPPLVPG FLAPPPPAPA QSPAPTQPPL PDAGVGELAP
101  DLLLEGIAED SIDSIIVAAS EQDSEIMDAN EQPQAKVTRS IVFVTGEAAP
151  YAKSGGLGDV CGSLPIALAA RGHRVMVVMP RYLNGSSDKN YAKALYTGKH
201  IKIPCFGGSH EVTFFHEYRD NVDWVFVDHP SYHRPGSLYG DNFGAFGDNQ
251  FRYTLLCYAA CEAPLILELG GYIYGQNCMF VVNDWHASLV PVLLAAKYRP
301  YGVYRDSRST LVIHNLAHQG LEPASTYPDL GLPPEWYGAL EWVFPEWARR
351  HALDKGEAVN FLKGAVVTAD RIVTVSQGYS WEVTTAEGGQ GLNELLSSRK
401  SVLNGIVNGI DINDWNPTTD KCLPHHYSVD DLSGKAKCKA ELQKELGLPV
451  REDVPLIGFI GRLDYQKGID LIKMAIPELM REDVQFVMLG SGDPIFEGWM
501  RSTESSYKDK FRGWVGFSVP VSHRITAGCD ILLMPSRFEP CGLNQLYAMQ
551  YGTVPVVHGT GGLRDTVETF NPFGAKGEEG TGWAFSPLTV DKMLWALRTA
601  MSTFREHKPS WEGLMKRGMT KDHTWDHAAE QYEQIFEWAF VDQPYVM*
```

FIGURE 21

Soluble Starch Synthase Genomic Clones



Probed with SM-2 full length cDNA

FIGURE 22

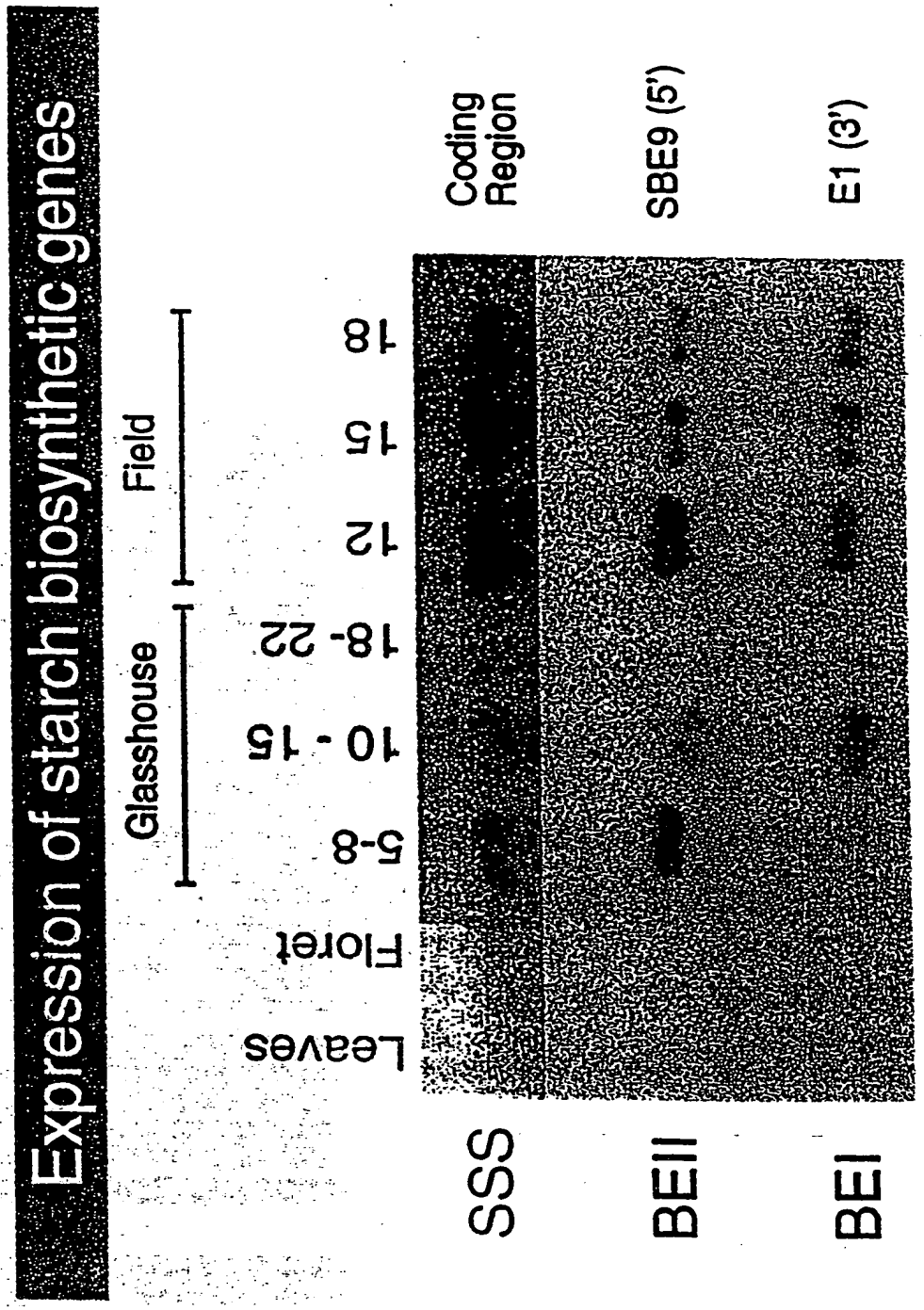

Expression of starch biosynthetic genes
Glasshouse
Field
Leaves
Floret
5-8
10 - 15
18- 22
12
15
18
Coding Region
SBE9 (5')
E1 (3')
SSS
BEII
BEI

FIGURE 23

Wheat DNA probed with
Soluble Starch Synthase

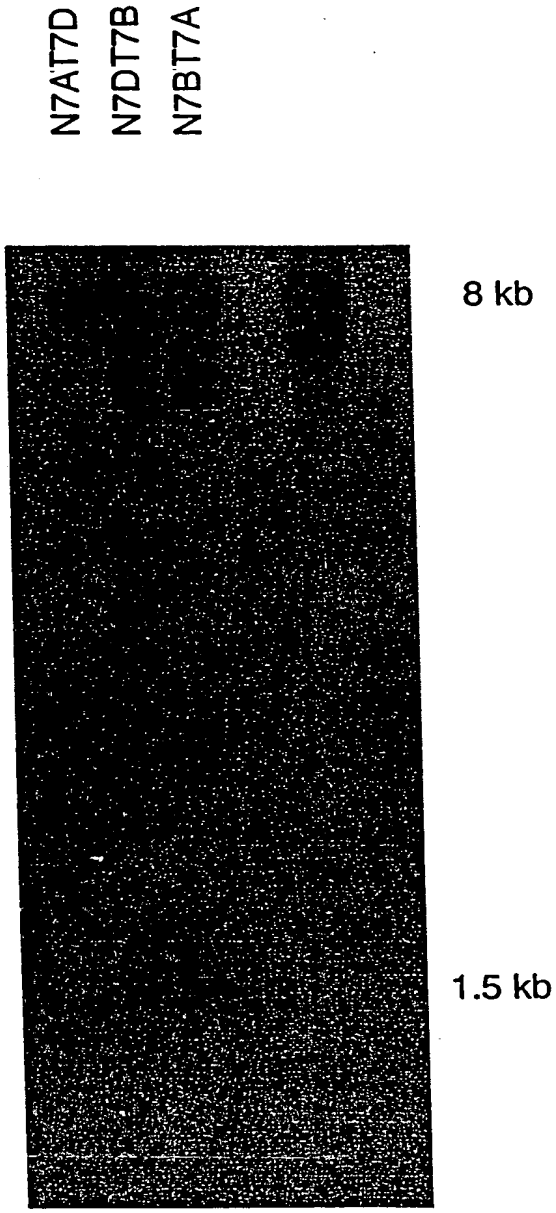


FIGURE 24

```
   1  GAGCTCCGAG AAnAGATTCC TATCATCGTC TTGGTGAGGT GAGGTTATGG
  51  TTTCTTGTCA TGTGGGCAGA TTTGGTGCCA GATGCTTCAT ATCTATTCAA
 101  GGGTTCAGCG GCAACAACTG CGGCTCCAGA GCGATGGTCC TTAAGGGCAC
 151  GTGCACGAAG ACTTCACGGC TGTTATCGAC AAGGTCAAGC CGGCTCCGAT
 201  AGGGGAGCAG CGACAGCGGC GCGTCAACCG CTCGTTCTGG CGGCAGTAGT
 251  GGTCGTTCGG TGCTCTCGGA ACCTCGATGT AATTTTTATG ATTTTAGAGA
 301  TGCTTTGTAc TTCcGATCGa TGAACTCTGA TAATAGATAT CTcTTCTcTc
 351  GCAAAAAAaG aGAGTTTTCA AcTGAAAACA AAaGaGTTTC AcTAGTTCTT
 401  CTTTTAGAAA CAGAGTTTCA cTAGCAcTTT TTTTTGcGAG AAGTcGAGTT
 451  TCAcTAAGTA cTAAaCCCAC GCAaTTATTC TCAAAAAAAA AACCCAcGcA
 501  ACTGTcTGgA TcCATCTTCG TTTTTTCCCC GAGAATCGTC TGgATcCATT
 551  TTCGTGTGCG AgGCATCCTC TCATTTTGcA cGgcCcAGcT cTcTTcTcGC
 601  CGGcGTAcGc TGctAcATgT cGgcAcTCcA cGCAAACAAA AaGAaGCCCA
 651  ACCGAAAAcG cAcGcGCcTT TcCAgGcTCA ccACGGaAAA AAaTACcAcG
 701  cGCcGcTcAC GAgCAAACCG TgACAACAGC CAGCCAGATA TGGCAACGGA
 751  GGcACGGGCC GcACACAGCC AcTGAAAACC GCAGcTGcTC TTCCGTCCGT
 801  CCGTCCcTCC GCCCGTCCGC gCcAcTCCAc TCGCCTTGCC CCAcTCCCAc
 851  TCTTCTCTCC CCGCGCACAC CGAGTCGGCA CCGGCTCATC ACCCATCACy
 901  TCGGCcTCGG CCACCGGCAA ACCCCCCGAT CCGCTTTTGC AGGCAGCGCA
 951  CTAAAACCCC GGGGAGCGCG CCCCGcgg.C AGCAGCAGCA CCGCAGTGGG
1001  AGAGAGAGGC TTCGCCCCGG CCCGCACCGA GCGGGGCGAT CCACCGTCCG
1051  TGCGTCCGCA CCTCCTCCGC CTCCTCCCCT GTCCCGCGCG CCCACACCCA
1101  TGG
```

FIGURE 25

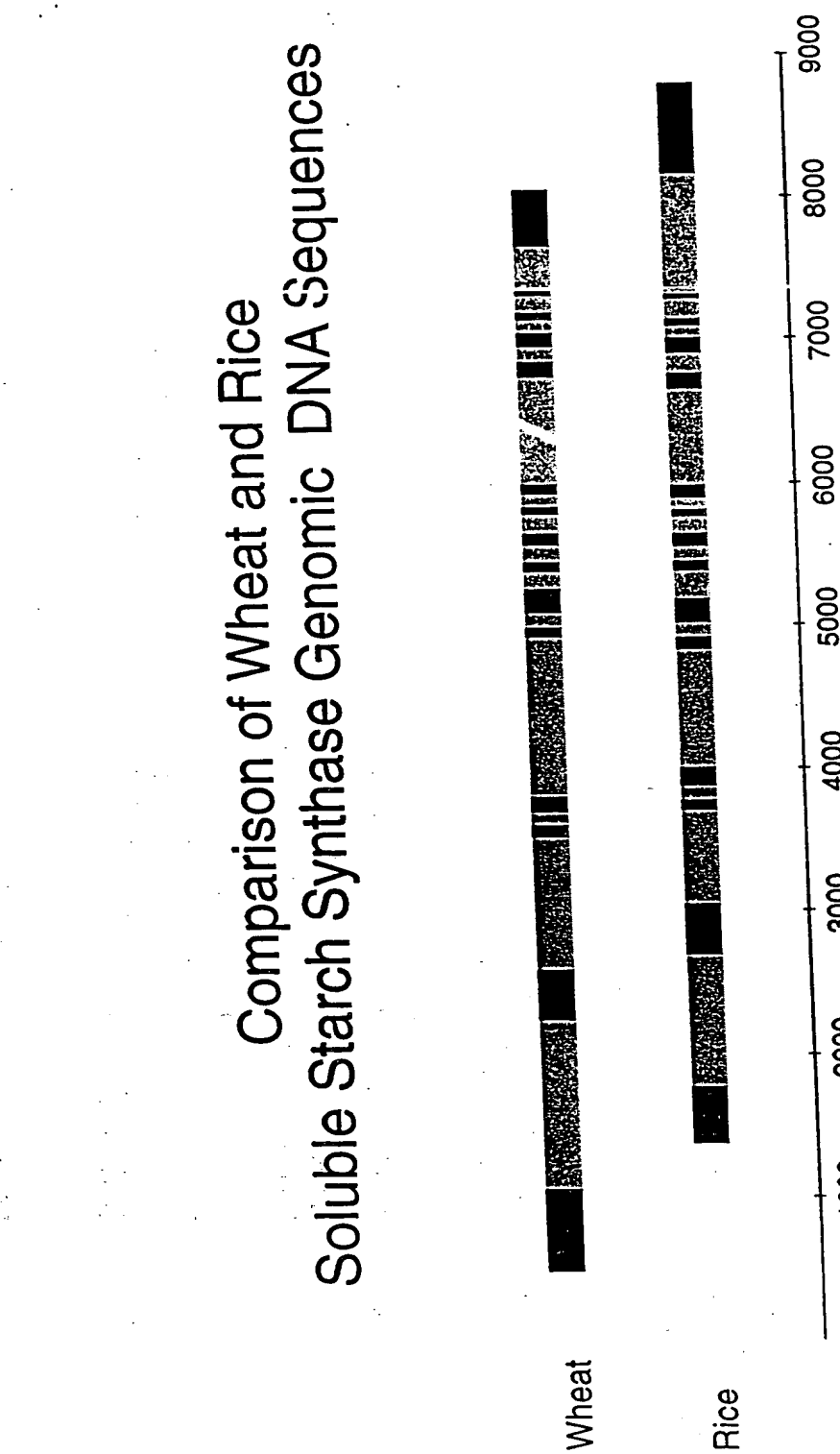
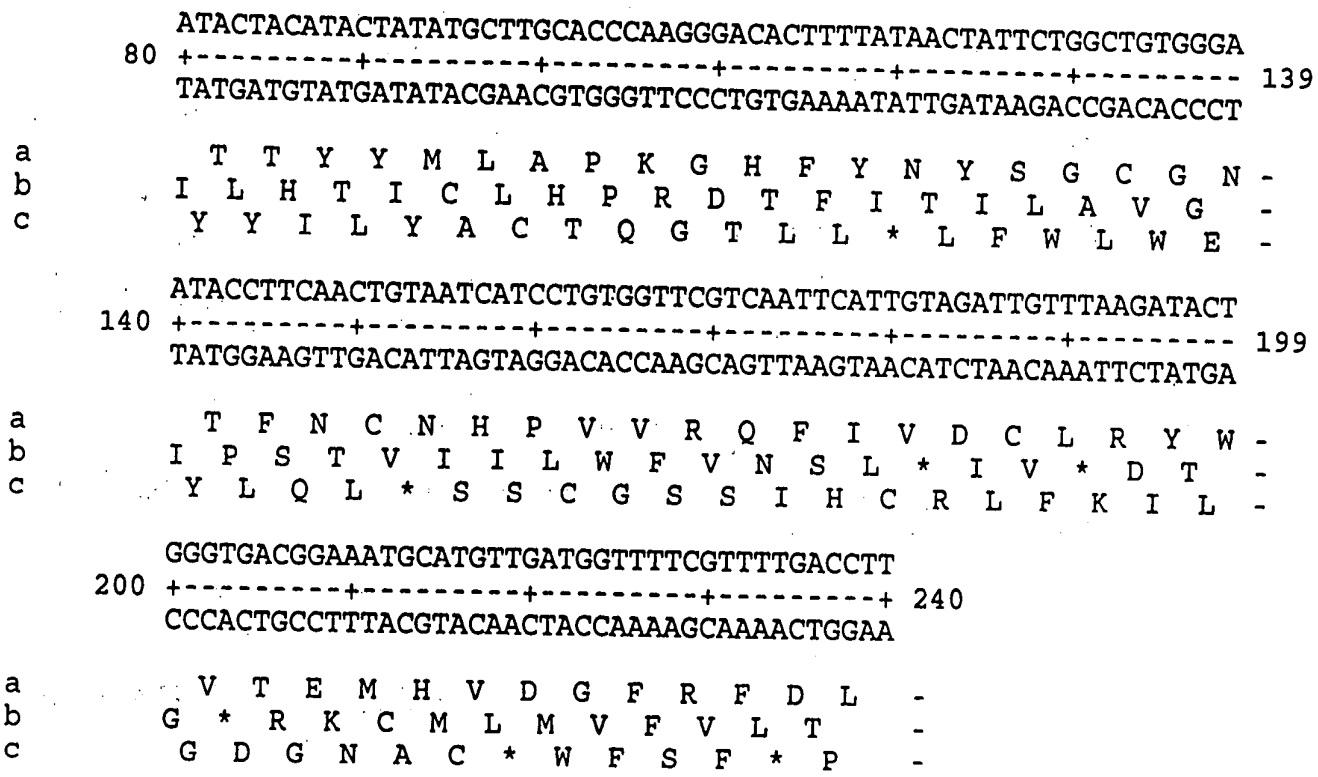

Comparison of Wheat and Rice
Soluble Starch Synthase Genomic DNA Sequences
Wheat
Rice
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
Base Pairs

FIGURE 26



Enzymes that do cut:

NONE

Enzymes that do not cut:

EcoRI

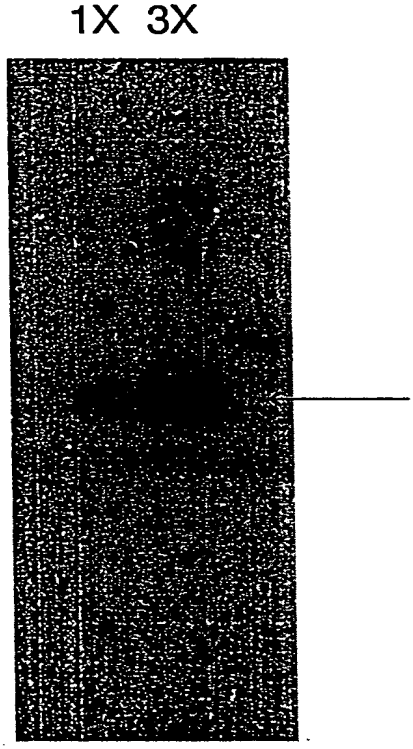
FIGURE 27a

Comparison of Wheat Debranching Enzyme-I (WDBE-I) PCR fragment with maize *Sugary*-1 DNA sequence

```
                1098      1107      1117      1127      1137      1147      1157
SUGARY.DNA        TGAGGTGATCATGGATGTTGTCTTCAATCATACAGCTGAAGGTAATGAGAAAGGCCCAAT
                  ||||||||||||||||||||||| |||||||||| |||||||||| || |||||
WHEAT1.DNA        ....GTGATCATGGATGTTGTCTTCAACCATACAGCTGAGGGTAATGAGAATGGTCCAAT
                   -3        6        16        26        36        46        56

FILE NAME      1158      1167      1177      1187      1197      1207      1217
SUGARY.DNA        ATTATCCTTTAGGGGGATAGATAATAGTACATACTACATGCTTGCACCTAAGGGAGAGTT
                  |||||| ||||||||| | ||||||| ||||||||| |||||||||| |||||| ||
WHEAT1.DNA        ATTATCATTTAGGGGGGTCGATAATACTACATACTATATGCTTGCACCCAAGGGACACTT
                  57        66        76        86        96        106       116

FILE NAME      1218      1227      1237      1247      1257      1267      1277
SUGARY.DNA        TTATAATTATTCTGGTTGTGGAAATACCTTCAATTGTAATCATCCTGTAGTCCGTGAATT
                  |||||| |||||||| |||||  |||||||||| |||||||||||||| || ||| ||||
WHEAT1.DNA        TTATAACTATTCTGGCTGTGGGNATACCTTCAACTGTAATCATCCTGTGGTTCGTCAATT
                  117       126       136       146       156       166       176

FILE NAME      1278      1287      1297      1307      1317      1327      1337
SUGARY.DNA        TATAGTGGATTGCTTGAGATACTGGGTAACAGAAATGCATGTTGATGGTTTTCGTTTTGA
                   || || ||||| || || |||||||| || |||||||||||| ||||||||||||||||
WHEAT1.DNA        CATTGTAGATTGTTTAAGNTACTGGGTGACGGAAATGCATGTTGNTGGTTTTCGTTTTGA
                  177       186       196       206       216       226       236

FILE NAME      1338      1347      1357
SUGARY.DNA        CCTTGCATCTATACT-G...
                  |||||||||| ||
WHEAT1.DNA        CCTTGCATCTN--CTTNAAA
                  237       246       256

MATCHING PERCENTAGE
      TOTAL WINDOW          84%     (  219/  260)
      ALIGNMENT WINDOW      86%     (  219/  253)
```

FIGURE 27b

Southern blot of T. *tauschii*
Genomic DNA



T. tauschii Genomic DNA Probed
With The Wheat Debranching Enzyme
PCR Product

FIGURE 28

Sequences of Primers which Direct PCR amplification of WSBEII-D1 introns

Intron	Foward primer	Foward primer Seq	Reverse primer	Reverse primer Seq	Predicted Length of Product
1	sr854.1180F	CTG GCT GAC TCA ATC ACT ACG	WSBE9E2R	GGC ACG AGT GTG TGT ACC TGT A	601
2	WBE2E1F	CGT CGC TGC TCC TCA GGA AG	WBE2E2R	CAG GAC CTT CCC TGG AGA GG	401
3	WBE2E2F	CGC AAC CTG AAG AAT TAC AG	sr866F	TAT CTT CAG GTA TCT ACA GC	309
4	WBE2E3F	ATT TTC GGA GCC ATC TTG AC	WBE2E4R2	ATG CTT CCA ATC CAC CTT CA	>450
5	WBE2E4F	TCG TGG TTA TGA AAA GCT TGG	WBE2E5R	GAG CCC ATT CTC GGT AAG TGA	234
6	sr913F	ATC ACT TAC CGA GAA TGG G	WBE2E6R	CTG CAT TTG GAT TCC AAT TG	232
7	WBE2E6F	ACA ATT GGA ATC CAA ATG CA	WBE2E7R	GGG AGG AAA ATC TCC CAA AC	402
8	WBE2E7F	AGC TAT TCC TCA TGG CTC AC	sr915F	CCA TTG AAA GGT ATT TCA CC	203
9	WBE2E8F	TGC AGG CTC CAG GTG AAA TA	sr912F	TAA CTT ATT GAC ATA CCG G	439

FIGURE 29

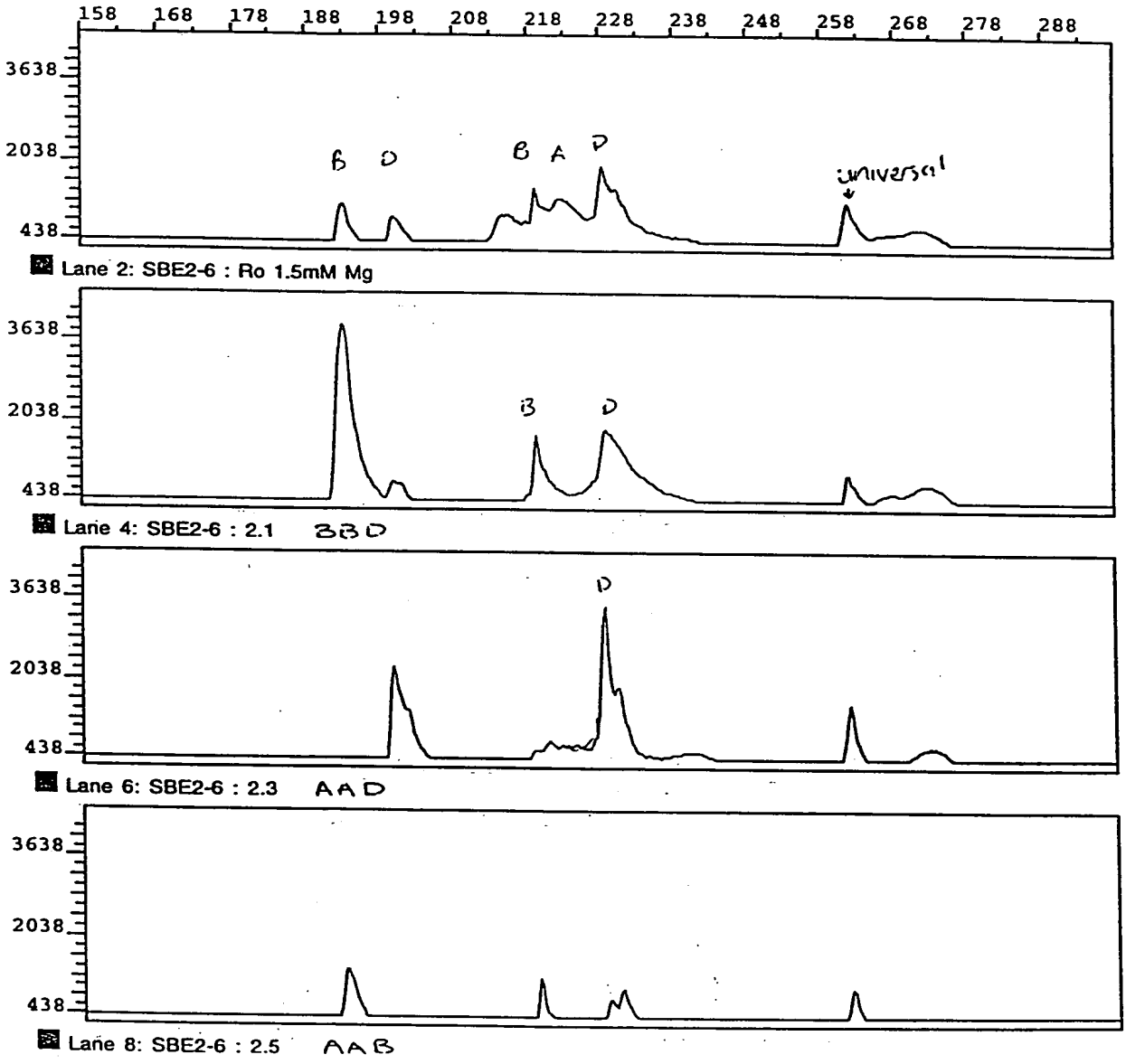

158 168 178 188 198 208 218 228 238 248 258 268 278 288
3638
2038
438
B D
B A D
universal
Lane 2: SBE2-6 : Ro 1.5mM Mg
3638
2038
438
B D
Lane 4: SBE2-6 : 2.1 BBD
3638
2038
438
D
Lane 6: SBE2-6 : 2.3 AAD
3638
2038
438
Lane 8: SBE2-6 : 2.5 AAB

FIGURE 30

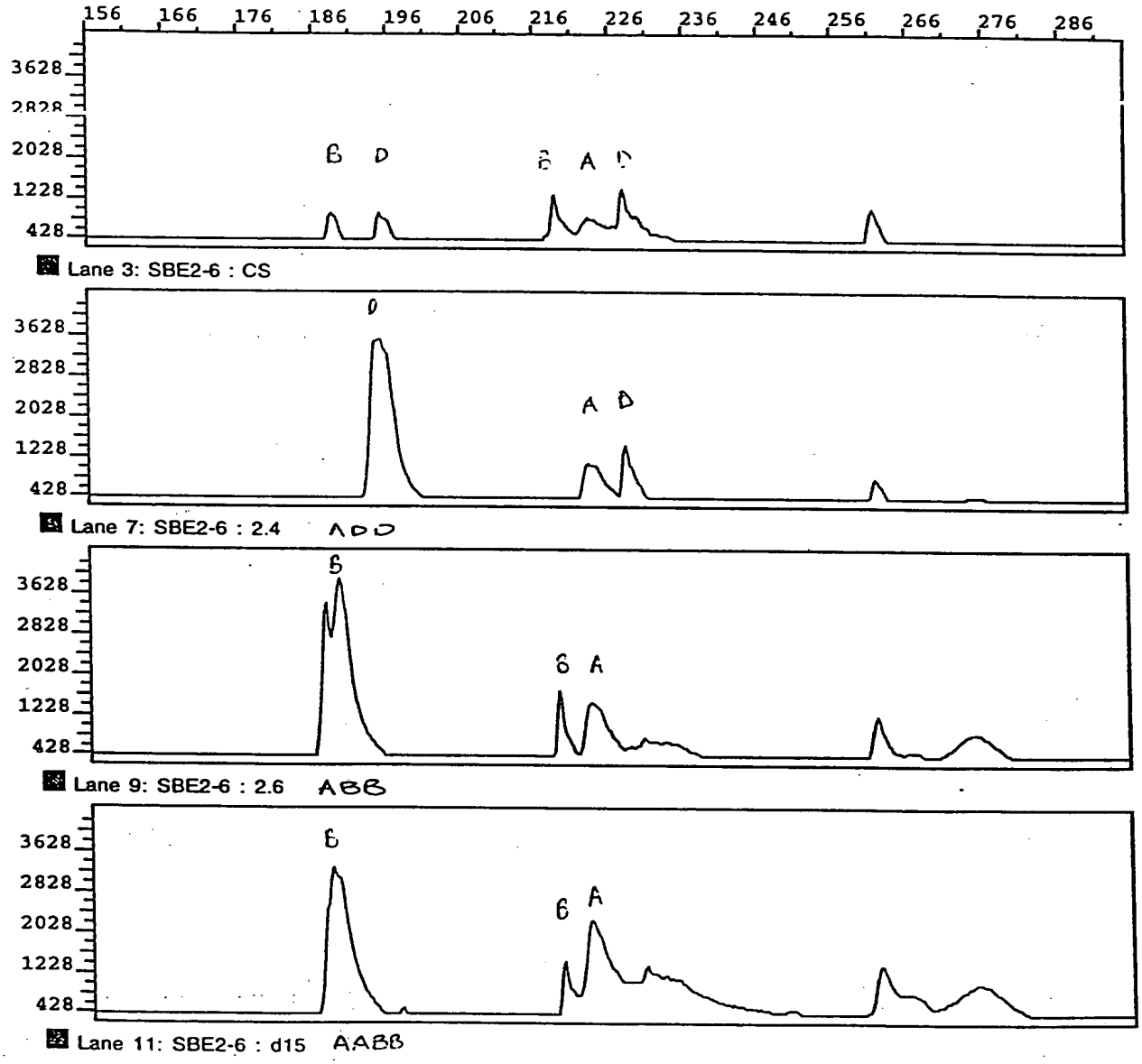


FIGURE 30 (cont.)

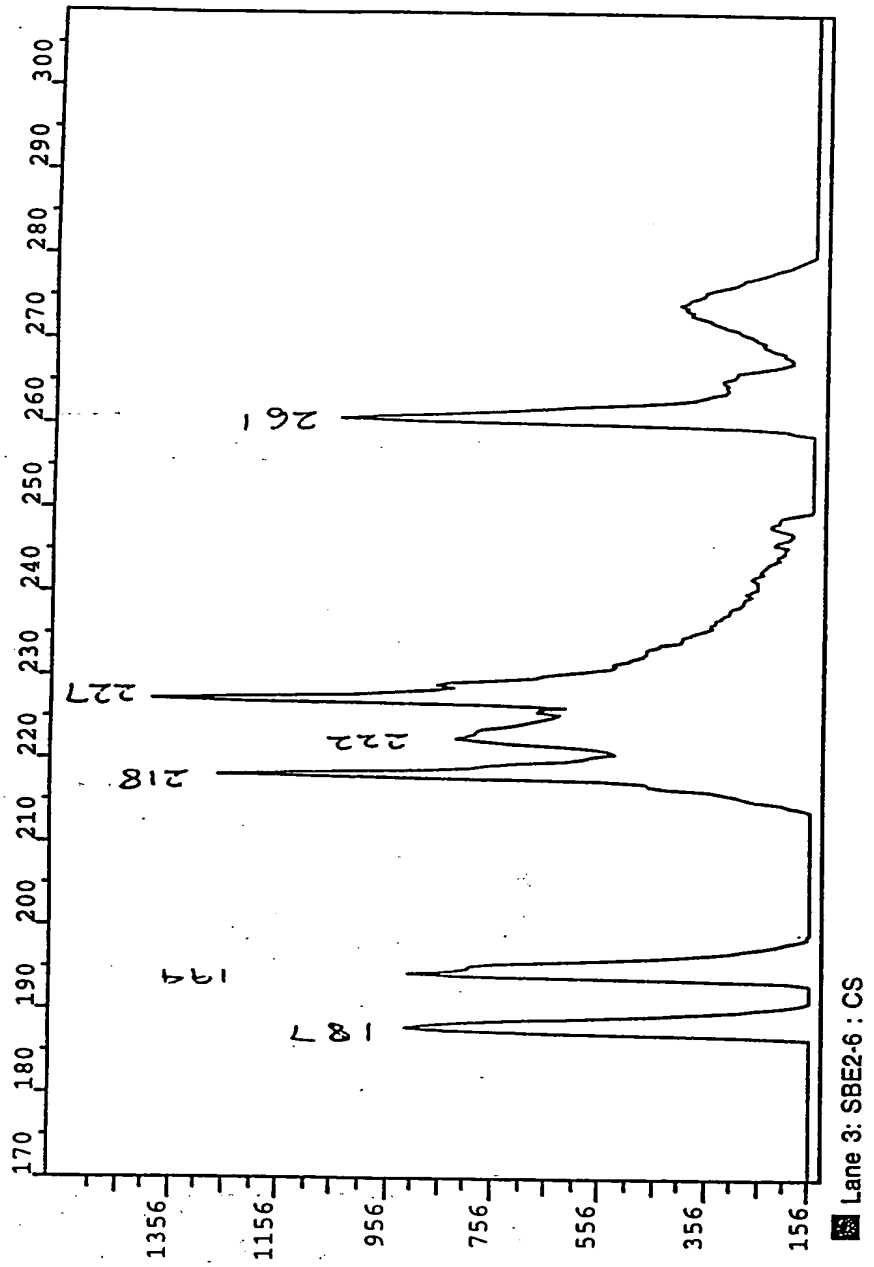

170
180
190
200
210
220
230
240
250
260
270
280
290
300
1356
1156
956
756
556
356
156
187
194
218
222
227
261
Lane 3: SBE2-6 : CS

FIGURE 31a

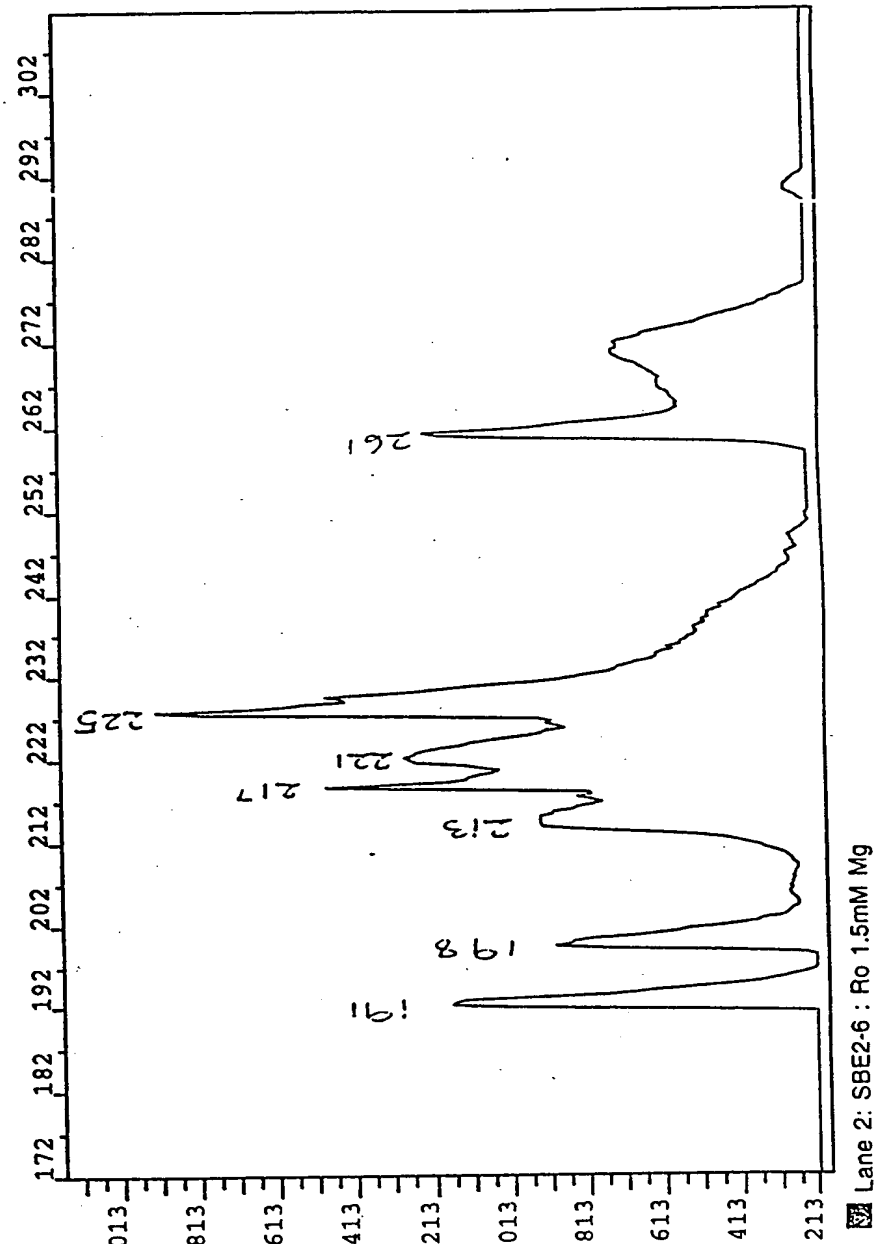

172
182
192
202
212
222
232
242
252
262
272
282
292
302
2013
1813
1613
1413
1213
1013
813
613
413
213
225
221
217
213
198
191
261
Lane 2: SBE2-6 : Ro 1.5mM Mg

FIGURE 31b